TEXTBOOK

of

BACTERIOLOGY

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PREFACE TO THE FOURTEENTH EDITION

The present edition of this book is more than a revision in the usual sense for in it the process of rewriting, begun in the preceding edition, has been continued. The chapters on the yeasts, the molds and the actinomycetes, previously only brought up to date, have been replaced by a completely new and relatively long chapter on medical mycology. Similarly, the chapter devoted to the parasitic protozoa has been replaced by a longer chapter on medical parasitology which includes the flukes, tapeworms and roundworms in addition to the protozoa. With minor exceptions the illustrations are completely new and, in the great majority of cases, original. The writer is indebted to his colleagues for the remainder, many of whom have generously allowed access to hitherto unpublished material for the preparation of photomicrographs for present purposes. Maps showing the geographical distribution of a number of the infectious diseases have been prepared or redrawn for this book and are also included.

In the process of revision a good deal of new material has been added. There are a number of new sections, including those on the respiratory enzymes and oxidation-reduction potential, the antibiotic substances, the mode of action of the sulfonamide drugs, iso-antigens and antibodies, bacteriological diagnosis of the various infectious diseases, and the sections on pinta, primary atypical pneumonia, postvaccination hepatitis, and epidemic kerato-conjunctivitis. Much material has been largely rewritten, such as the chapter on Asiatic cholera, the sections on the virus diseases of man, and especially those on influenza and encephalitis, and the sections on the pleuropneumonia group of microorganisms, the antigenic structure of the *Salmonella* types, experimental epidemiology, and the more general considerations of the rickettsiae. A number of tables have been added throughout the book which supplement or summarize material in the text.

The order of presentation has been altered slightly so that the various microorganisms are taken up in somewhat more logical sequence. The cholera vibrio, for example, has been removed from proximity to the spirochetes and included with the enteric bacilli, the chapter on *Rickettsia* has been moved to precede rather than follow the filterable viruses, the pleuropneumonia group removed from the viruses and placed with *Bartonella*, etc.

The writer is greatly indebted to his colleagues for invaluable assistance in large and small ways. Dr. Richard J. Porter has undertaken full responsibility for the chapter on medical parasitology, and has not only written it but also has included a number of original drawings. Dr. F. B. Gordon has revised his chapters on the viruses, and these, and the chapter on medical parasitology, are specifically credited to him and to Dr. Porter, respectively. Dr. Selman A. Waksman, Dr. Eleanor M. Humphreys, Dr. Stephen Rothman and Dr. R. Wendell Harrison have read the manuscript

of the chapter on medical mycology and have given valuable criticism. Dr. Harrison has also revised the section on *Lactobacillus* and dental caries. Very many illustrations of the mycoses were made possible through the kindness of Dr. Humphreys; in a number of instances her cooperation has included the cutting and staining of new sections for this purpose. Dr. Rothman has allowed the writer free access to his files of photomicrographs of the pathogenic fungi. Dr. W. H. Taliaferro, Dr. Theodor Rosebury, Dr. A. B. Sabin, Dr. S. E. Luria, Dr. R. L. Starkey and Dr. Waksman have been most generous with original illustrations. The writer is especially indebted to Mr. Jean W. Crunnelle of the Photographic Department of the University of Chicago whose skill and patience have made possible very many of the illustrations. To these and the many others who have offered valuable and constructive criticism the writer is deeply grateful and appreciative.

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CONTENTS

CHAPTER 1

THE HISTORY AND DEVELOPMENT OF BACTERIOLOGY[1]

Bacteriology illustrates, perhaps better than any other branch of biology, the complex system of interrelationships among the sciences. Since the microorganisms are so small that they are invisible not only to the naked eye but to the eye aided by simple lenses, their very discovery necessarily depended upon the development of optical science to such a point that lenses of sufficient perfection and magnifying powers became available. It is not strange, therefore, that the many evidences of bacterial activity, such as the putrefaction and decay of organic matter, the infectious diseases of man and lower animals, the common fermentations and similar natural phenomena, should have been viewed in the past as semimetaphysical processes. Despite the entire lack of evidence, some of the older writers voiced a belief in the existence of such microorganisms. Fracastorius of Verona (1546) suggested a *contagium vivum* as a cause of disease, and von Plenciz (1762) accounted for the specificity of disease on the basis of microbic etiology. Although such speculations might be considered a result of prophetic insight, in all probability they were no more than guesses to which, in view of present knowledge, it is not difficult to ascribe significance. In the absence of the experimental approach, the fertility of philosophic speculation remains a doubtful quality.

Kircher had reported the observation of "minute worms" in the blood of plague patients in 1659, but it is doubtful that he ever saw the plague bacilli. The first concrete foundations of bacteriology were laid by a Dutchman, Antonj van Leeuwenhoek, in the latter part of the seventeenth and the early part of the eighteenth centuries. Leeuwenhoek, a man of indefatigable industry and great curiosity, held a political sinecure in his native town of Delft. In his odd time, of which he apparently had a good deal, he ground lenses and became the most skilful lens grinder of his time. He utilized these lenses in the construction of simple microscopes and spent many years of his long life (1632–1723) in examining a great variety of natural objects, with unremitting industry if without system, and in the course of his observations chanced to come across the organisms now known as bacteria. That he did in fact observe these creatures is evident from the drawings he left of them. In a communication to the Royal Society,[2] of which he was a member, he states, "Having several times endeavoured to discover the cause of the pungency of pepper

[1] For details see Bulloch: *The History of Bacteriology*. Oxford University Press. New York. 1938.

[2] Phil. Trans. Roy. Soc., 1677, *11–12*:821. Observations, communicated to the publisher by Mr. Antony van Leeuwenhoek, in a Dutch letter of the 9th of Octob. 1676. Here English'd: Concerning little animals observed by him in rain- well- sea- and snow water, as also in water wherein pepper had lain infused.

upon our tongue, and the rather, because it hath been found, that though pepper had layn a whole year in vinegar, yet it retained still its pungency; I did put about $\frac{1}{3}$ of an ounce of whole pepper in water, placing it in my study, with this design, that the pepper thereby being rendered soft, I might be enabled the better to observe what I proposed to myself. This pepper having lain about three weeks in the water, to which I had added some snow water, the other water being in great part exhaled, I looked upon it the 24th of April, 1676 and discern'd in it, to my great wonder, an incredible number of little animals, of divers kinds; . . . The 4th sort of creatures, which moved through the three former sorts, were incredibly small, and so small in my eye, that I judge, that if 100 of them lay one by another, they would not equal the length of a grain of coarse sand; and according to this estimate, ten hundred thousand of them could not equal the dimensions of a grain of such sand." Leeuwenhoek is known to have used only simple lenses, and the highest magnification he reached was approximately 300 diameters. Probably he did not observe objects as small as bacteria by transmitted light. He kept his method of illumination secret; it is not improbable that he used reflected light, similar to that used in present day ultramicroscopes. Although his observations did not lead to an immediate development of bacteriology, their significance was apparent to many of the scientific men of the day. Slare, in a comment on a report[3] on an epidemic among cattle, concludes with the remark, "I wish Mr. Leeuwenhoeck had been present at some of the dissections of these infected Animals, I am perswaded he would have discovered some strange Insect or other in them."

Nearly a century later, in 1786, the Danish zoologist, O. F. Müller, studied these microorganisms and succeeded in discovering many structural details of which his predecessors had been ignorant. He depicted several kinds of bacteria so accurately that they can be identified today as belonging to one or another of the chief divisions.

Another unequivocal advance was made by Ehrenberg (1795–1876). His principal work upon the "infusion animals" or "Infusionstierchen," as these organisms were then termed, was published in 1838 and brought together much more definite and detailed information concerning bacteria than had been previously secured. The chief merit of Ehrenberg's work lay in the system that it introduced into the study of microorganisms. He was able to establish a number of different groups among the organisms now known as bacteria, and recognized clearly the fundamental differences between the larger forms, such as the screw-shaped or spirally-twisted organisms, and certain of the true protozoa with which they had heretofore been classed. Some of the names which Ehrenberg conferred upon his "infusion animals," such as bacterium and spirillum, are still current in bacteriological nomenclature, although with changed signification.

[3] Phil. Trans. Roy. Soc., 1682, *13*:93. An abstract of a letter from Dr. Wincler, chief physitian of the Prince Palatine, Dat. Dec. 22. 1682 to Dr. Fred Slare, Fellow of the Royal Society, containing an account of a Murren in Switzerland, and the method of its cure. A further confirmation of the above mentioned Contagion, of its nature, and manner of spreading by way of Postscript from the ingenious Fred Slare, M. D. and F. R. S. Dat. March 27, 1683.

In the two or three decades succeeding Ehrenberg's work considerable knowledge was amassed concerning the mode of development and physiology of bacteria, as well as their position in biological classification; but the labors of Dujardin, Perty, Cohn, Nägeli and others, although important, are quite overshadowed by the work of Louis Pasteur (1822–1895).

Up to the period of Pasteur's investigations the rôle played by bacteria in various familiar natural processes, such as putrefaction, decay and fermentation, had been, perhaps, vaguely suspected, but had not received conclusive demonstration. Pasteur, originally trained as a chemist, had done his early work on stereoisomerism. The formation of optically active amyl alcohol during the course of the lactic acid fermentation attracted him to the study of the fermentation processes. The demonstration of the plant-

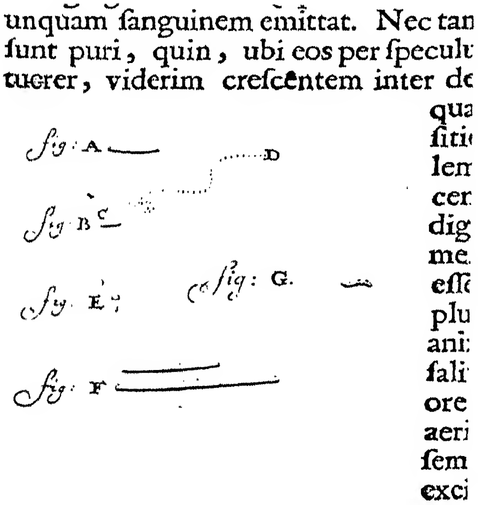


Fig. 1.—The first pictorial representation of bacteria. Reproduced from *Arcana Naturae delecta ab Antonio van Leeuwenhoek.* Delphis Batavorum apud Henricum Crooneveld. 1695.

like nature of yeast by Caignard-Latour and by Schwann, together with the asymmetric synthesis of amyl alcohol, led him to suspect that fermentation was a result of the activity of living cells. This view was strongly opposed by the chemists of the day, Liebig, Berzelius and Wöhler in particular, who regarded the presence of dead and dying yeast cells of importance only in that in the course of their own molecular disintegration they toppled over and dragged down certain complex organic molecules with which they were in contact. Pasteur's researches led him deeper into the morass of fundamental biology than, perhaps, he had anticipated, for, before the essentially biological basis of fermentation could be conclusively demonstrated, the problem of spontaneous generation of life from decomposing organic materials presented itself for solution.

For a great many years it had been generally held that living things, even organisms as large and complex as mice, were spontaneously generated during the course of the decomposition of organic substances. The experiments of the poet physician Redi (1626–1697), however, indicated definitely that maggots were not spontaneously formed from decomposing meat but were in fact fly larvae hatched from eggs deposited in the meat by flies. Spallanzani, an Italian monk, showed further that putrescible meat infusions, when properly heated, did not spoil and did not contain living organisms even though kept over long periods of time. Needham, an Irish priest, took issue with Spallanzani on the basis of similar experiments in which spoilage took place and living organisms appeared in spite of previous heating. A second series of elaborate experiments by Spallanzani corroborated his earlier findings and indicated the fallacies in Needham's experiments. Other experiments by Schulze, Schwann, Schröder and von Dusch indicated that no spontaneous generation took place but rather that the source of living organisms was the air in which they were suspended, a conclusion amply confirmed by the careful experiments of the Englishman, Tyndall. The entire controversy was revived by the extended experimental work of Pouchet, which appeared to indicate a spontaneous generation of microorganisms in heated organic materials; and Pouchet created a great stir in French scientific circles—a stir in which Pasteur found himself.

The point at issue was, obviously, whether spoilage of such infusions took place as a result of the presence of microorganisms or whether the appearance of living organisms was a result of the decomposition—a general question of which the question of the biological basis of fermentation was a special case. Pasteur's extended and careful investigations showed beyond reasonable doubt that microorganisms were present in air, the numbers varying with the place from which the air came; that putrescible material which had been heated sufficiently to destroy all life would keep indefinitely with no evidences of either decomposition or the presence of living organisms; and, finally, that a common source of contamination of such sterilized material was air in which living microorganisms were suspended.

The fact that life came only from life (biogenesis) and was not spontaneously generated from non-living materials (abiogenesis) having been established,[4] it was not difficult to prove that fermentations resulted from the physiological activities of living, growing microorganisms. Furthermore, the specificity of fermentations, the fact that different kinds of fermentations were consequences of the activities of different kinds of microorganisms, grew out of his work. The transition from studies on fermentation to studies on infectious disease was not a difficult one to make, for there was a growing awareness among scientific men of the day of the similarities between the development of disease in an individual and the fermentation of sugar solutions.

Pasteur was led into a study of the "diseases" of beer and wine, proc-

[4] Speaking scientifically. Philosophic speculation is beyond the scope of the present volume.

esses which he found to be none other than secondary fermentations brought about through the activities of extraneous microorganisms, decompositions that resulted in the accumulation of end products of an undesirable nature. He controlled the fermentative process by gentle heating followed by inoculation of the fermentable mixture with microorganisms which brought about the desired type of decomposition. The heating process has been termed "pasteurization" in his honor. A further development was a study undertaken at the request of the silk growers, of pebrine, a disease of silk worms which was assuming considerable economic importance. Here too a microbic etiology was discovered and practical control measures could be and were applied.

The so-called germ theory of disease had, at this time, permeated more and more generally into the scientific thought of the day, largely, perhaps, as a result of the strong support it received from Pasteur's researches on fermentation. Lister (1827–1912), an English surgeon, was one of the first to grasp the significance of Pasteur's work in relation to human disease, and put the new concepts into practical operation with his introduction of the antiseptic technique into surgical operations. The free use of strong carbolic acid brought about a marked decline in surgical mortality, which had been terrifically high in even the most minor operations. It remained, however, for Robert Koch (1843–1910), a German physician, to develop the experimental methods necessary to the proof of the causal relation between bacteria and infectious disease.

One of the greatest difficulties encountered by the early workers in bacteriology was that of separating bacteria from one another. That morphologically different types existed was clear; and, further, that organisms exhibiting the same morphology differed from one another in ability to produce disease had been apparent from the early work on pyemia. Perhaps one of the greatest single contributions to bacteriology is the method of isolation of bacteria in pure culture developed by Koch. It consisted, essentially, of culture on a semisolid medium, a nutrient environment solidified by the addition of gelatin or agar-agar—a method so simple and yet so effective that it is used practically unchanged today (p. 21). Koch had achieved what he had said only a year or two before was "impossible."

Although it was almost entirely through the works of Pasteur that bacteria and other microorganisms emerged from their relative obscurity as organisms of interest chiefly to the professional biologist and took a conspicuous position in natural science as a group of organisms whose activities and capabilities were full of a far-reaching significance for all mankind, the contributions of Koch and the German school were also of fundamental significance. If any one man can be looked upon as the founder of bacteriology, that man is Louis Pasteur. Likewise, the infant science owed its firm experimental foundation directly to Koch, a foundation without which it could never have become a science.

The isolation of bacteria in pure culture, coupled with the methods of staining developed by Koch, Ehrlich, Wiegert and others, so markedly stimulated the study of bacteriology that the ensuing decade or two,

the eighties and nineties, became in truth the first golden age of that science. As the history of all science is, in essence, the history of its methodology, so in bacteriology discovery of the specific etiology of a variety of infectious diseases followed rapidly upon Koch's studies of anthrax and his isolation of the anthrax bacillus. Koch himself isolated and described the tubercle bacillus in 1882 and the cholera vibrio the following year. Other discoveries tumbled in one after another with bewildering rapidity. Klebs described the diphtheria bacillus in 1883, and in 1884 Löffler isolated and studied this organism; Fraenkel discovered the pneumococcus in 1886, and the following year Weichselbaum isolated the meningococcus; Kitasato cultivated the tetanus bacillus in 1889, and in 1894 he and Yersin discovered the plague bacillus independently. These and many other similar discoveries made clear to the world in a striking fashion the significant implications of the new science.

Meanwhile, the investigations of the French workers had taken a somewhat different turn. Many years before, in 1796, Jenner, an English physician, observed that infection of human beings with cowpox, a disease of cattle closely resembling smallpox, protected against subsequent infection with smallpox. Pasteur, investigating first chicken cholera and later anthrax and swine erysipelas, succeeded in demonstrating a basic principle of immunology, that inoculation with attenuated microorganisms, those which by some sort of treatment had lost their virulence, resulted in the development of an increased resistance or immunity of the inoculated individual against later infection with the same organism. Pasteur's most striking application of this principle was in the development of a prophylactic treatment against rabies—an accomplishment with some elements of the dramatic because of the dread in which the disease was held. Not long afterward the American workers, Salmon and Theobald Smith, discovered that the inoculation of killed bacteria would also stimulate the development of the immune state. The cellular theory of immunity propounded by the Russian zoologist, Metchnikoff, was soon overshadowed by the discovery of tetanus antitoxin by von Behring and Kitasato and, shortly afterward, the discovery of diphtheria antitoxin by von Behring, and the precise studies of the humoral antibodies by Bordet and others; only in recent years has it come into its own. The utilization of the immune phenomena in the diagnosis of disease by Wassermann, Widal and others, Paul Ehrlich's development of his side-chain theory of immunity and his initiation of chemotherapy through the synthesis of salvarsan all contributed to the expansion and development of bacteriology. The discovery of the filterable viruses, apparently living agents too small to be seen with the most powerful microscopes, by Iwanowski, Beijerinck, and Löffler and Frosch brought up new problems, many of which are still unsolved today.

The new science, sired by Pasteur and nurtured by Koch, expanded beyond medicine and infectious disease into agriculture and the industrial fields. The genius of the Russian worker, Winogradsky, made possible the elucidation of the perplexing problems of soil fertility by his isolation of autotrophic bacteria which oxidized ammonia to nitrites and nitrates.

The discovery of the nitrogen-fixing bacteria living symbiotically with leguminous plants by Hellriegel and Wilfarth and of the free-living nitrogen-fixing forms by Winogradsky and by Beijerinck further clarified the puzzles of soil fertility and indicated the important functions of the soil bacteria. The discovery of the bacterial etiology of pear blight by Burrill and the isolation of bacteria responsible for many other diseases of plants have shed new light on old agricultural problems. It has also been discovered that other kinds of bacteria impart the characteristic flavors or aromas to butter, cheese and other dairy products; and that still others determine the success or failure of various industrial processes, such as the retting of flax, the tanning of hides and, perhaps, the curing of tobacco.

Thus, in the space of only a few decades, bacteriology had become a full-blown, vigorous young science, fully capable of standing on its own feet—and this from Leeuwenhoek's "little animals" that behaved in such curious ways.

The outstanding success of the practical applications of bacteriology should not be allowed to overshadow the fact that it owes its present important place among the biological sciences quite as much to its general scientific significance. It has been often pointed out that bacteriology has produced a change in man's conceptions of the world around him so sweeping as almost to deserve the term "revolutionary." Up to the middle of the nineteenth century the character of many of the most familiar natural processes, such as decay, fermentation and the like, was entirely misunderstood; contemporary spontaneous generation of at least the lower forms of life was the generally accepted belief of most scientific men; infectious diseases were not sharply differentiated from one another and the most fantastic hypotheses were advanced to explain their existence. Although the great mass of material phenomena elsewhere had been brought into apparent orderliness and system, here was a region in which the unscientific imagination rioted in mystery and extravagance. The penetration of this realm of obscurity by the discoveries of bacteriology gave the human race for the first time in its history a rational theory of disease, dispelled the myths of spontaneous generation, and set the process of decay and kindred phenomena in their true relation to the great cycle of living and non-living matter. The new conception of the microscopical underworld which bacteriology brought into biological science must be reckoned as a conspicuous landmark, and, in so far as it has changed the attitude of man toward the universe, should be regarded as one of the most important triumphs of natural science.

Underlying all the applications of bacteriology are certain fundamental facts and principles concerning the structure, mode of development, and general physiologic requirements and capabilities of bacteria themselves. This subject matter constitutes the ground work of bacteriology, and is essential not only to a proper comprehension of the present practical applications of bacteriology, but also to the further development of the science.

CHAPTER 2

METHODS OF STUDYING BACTERIA

The various methods used in the study of bacteria are based upon intensive studies on the physiological capabilities and requirements of these organisms as well as their relation to environmental circumstances, not only favorable, but detrimental or lethal. Although the information gained from such studies is considered at greater length in subsequent chapters, a brief résumé of the commonly used methods is essential to the initiation of bacteriological studies.

In general, bacteria are studied in pure culture, *i.e.*, a single kind of bacterium free from other similar organisms. The isolation and maintenance of bacteria in pure culture obviously entails the elimination of extraneous microorganisms from the glassware, culture media, instruments and other pieces of equipment used in the manipulation and culture of these organisms. Bacteria and other fungi, such as yeasts and molds, are widely distributed over the world as we know it. So ubiquitous are these organisms that there are few places where they may not be found. The elimination of contaminating microorganisms consists, therefore, not in avoiding them, for this is ordinarily impossible, but in their destruction. Complete destruction of living material, or sterilization, as it is usually called, although not as simple as might be thought, is not difficult when the proper precautions are observed.

The Sterilization of Glassware and Instruments.—The agent most commonly used for sterilization is heat in one form or another. Dry heat, although not as efficient as moist heat, is a most convenient means of sterilizing pieces of equipment such as glassware and instruments. Glassware—flasks, Petri dishes, etc.—should be clean and dry and if new should have had a preliminary cleaning in sulfuric acid-dichromate cleaning solution. Containers such as flasks, test tubes and the like should be plugged with a good grade of non-absorbent cotton. The cotton plug may be folded and inserted into the flask or test tube with forceps or it may be rolled into the form of a plug. At times gauze plugs, cotton plugs wrapped in gauze, may be desirable. The presence of cotton plugs allows the free passage of air but prevents the entrance of microorganisms floating about in the air, thus maintaining sterility within the container after removal from the sterilizer. Petri dishes and pipettes, the latter usually plugged at the mouth end with cotton, may either be wrapped in paper or placed in closed cans. Surgical instruments may be wrapped in towels or paper; hypodermic needles are commonly placed in plugged test tubes; syringes may be separated and wrapped in paper; and other instruments may be similarly prepared. The plugged tubes and flasks, pipettes and Petri dishes and other pieces of equipment are placed without crowding

into a hot air sterilizer. These are ovens of one type or another, the Lautenschläger type being one of the most efficient. Such ovens are heated either by gas or electricity and are equipped with thermometers. The temperature is raised to 170° to 180° C. and maintained for a period of two hours. This suffices to destroy all bacteria, even the most resistant forms. There are two possible sources of error which may result in failure to destroy all microorganisms. First, the material may be too crowded or tightly packed in the oven, and, second, in some ovens considerable variation in temperature occurs in different parts, in some of which it may not be sufficiently high to insure sterilization. The latter, however, is not a source of error in ovens of proper construction. In the absence of exact temperature control, a slight browning of the cotton is sometimes taken as evidence that the proper temperature has been reached and maintained.

Some other instruments, for example the tips of forceps and the platinum or nichrome wire loops used for transferring bacteria, may be sterilized by heating to a dull red in the bunsen flame.

A more efficient means of destruction is moist heat. Simple boiling either in water or 1 per cent soda solution for three to five minutes suffices to destroy vegetative forms of most bacteria. When instruments are to be used for operative purposes, boiling suffices, since it destroys all pathogenic bacteria; but if they must be free from all bacteria, they must be sterilized in a hot air oven or by steam under pressure.

Intermittent Sterilization.—It was discovered many years ago by Tyndall that a single boiling did not destroy all microbic life, even though prolonged for some time, but that if the boiling was repeated on successive days, putrescible material could often be rendered sterile. The procedure of intermittent sterilization, occasionally called tyndallization, has since been found to be successful. The bacteria not destroyed in the initial heating are in the resistant spore stage; by the following day most, if not all of these, have germinated into the less resistant vegetative cells and can be destroyed by the second boiling. The method is useful in the sterilization of materials which would partially decompose under the influence of higher temperatures.

A more convenient as well as more efficient method than simple boiling is to expose the material to be sterilized to free-flowing steam. The Arnold sterilizer commonly used in the laboratory is constructed with a false bottom so that a minimum volume of water is heated to produce steam quickly, while the main tank is constantly fed by the water of condensation which is caught and collected by an outer jacket.

The medium or other material to be so sterilized is heated for fifteen to twenty minutes. The steaming process is repeated on the second and third days, the medium being kept at 20° C. in the intervals. This method of sterilization is not, however, always successful. Bacterial spores often show delayed germination, and the transition to the less resistant vegetative forms may not take place in the twenty-four-hour interval between steamings. Further, the spores of obligate anaerobes may not find sufficiently anaerobic conditions in the solutions and therefore may not ger-

minate until subsequently inoculated with bacteria that produce anaerobic conditions incidental to their growth processes. If, however, four successive steamings are employed and the interval between steamings is lengthened to forty-eight hours, most flasks of media will remain sterile.

Autoclave Sterilization.—By far the most efficient method of sterilization is the use of steam under pressure. The temperature of boiling water at 760 mm. Hg atmospheric pressure is 100° C., but if the water is confined so that the pressure within the container rises, the boiling point and, consequently, the temperature rise also. In this way it is possible to take advantage of the high sterilizing efficiency of moist heat at temperatures above 100° C.

The devices commonly employed for the use of steam under pressure include the common pressure cooker, autoclaves of various types and design, and the more complex "dressing sterilizers," which are so arranged that after sterilization a vacuum can be applied to dry cloth materials quickly and completely. The horizontal autoclave is more complex than the pressure cooker in that it is steam jacketed and is somewhat more efficient. Steam may be supplied either by connection to a high pressure steam line or by a gas or electrically heated steam generator usually mounted beneath the sterilizing chamber.

The autoclave is much used in bacteriological laboratories for the sterilization of all kinds of media, such as broths, semisolid media, milk, etc., as well as apparatus, test tubes, discarded cultures and the like. Autoclave sterilization is not practical for surgical instruments and similar things which may rust. In general, exposure to a pressure of fifteen pounds (120° C.) for fifteen minutes is sufficient to sterilize completely. Time and pressure are relative, however; tubed media in closely packed baskets should be kept at fifteen pounds for twenty minutes, and broths in liter quantities may require thirty minutes. In any case, the method is highly efficient, incomplete sterilization being an extremely rare occurrence.

A number of precautions must be observed in the operation of the autoclave. When steam is first admitted, the escape valve must be left open until all the air originally within the chamber has been displaced by steam. If air is allowed to remain, the pressure attained will be deceptive in that the temperature will not rise accordingly. After sterilization is complete, pressure must be reduced slowly. If it goes down too rapidly, media and other liquids will boil violently, not only blowing out the cotton plugs, but also boiling out most of the liquid. A satisfactory procedure is to shut off the steam and close all the valves, the pressure going down, therefore, only as rapidly as the autoclave cools off. If there are no leaks, this process usually requires ten to fifteen minutes.

Sterilization by Chemicals.—Sterilization by the use of germicidal chemical compounds is not common. At times, however, objects such as rubber tubing, rubber stoppers and the like which are adversely affected by heat may be sterilized by thorough cleaning in soap and water, followed by immersion in 1:1000 mercuric chloride solution for one hour. The bichloride may be removed before use by thorough washing with sterile water.

Sterilization by Filtration.—It is often desirable to sterilize water, sugar solutions and other fluids, such as liquid culture media in which bacteria have been growing, without subjecting them to heat or to the action of disinfectants. The method of sterilization by filtration is particularly useful in obtaining soluble bacterial products such as toxins and enzymes, which might be injured by chemicals or heat. It consists in passing the solution through a filter so fine that the bacterial cells are retained. The finest of filter paper is much too coarse to hold back bacteria and, consequently, special filters must be used. These are ordinarily in the form of cylinders closed at one end and made of infusorial earth or

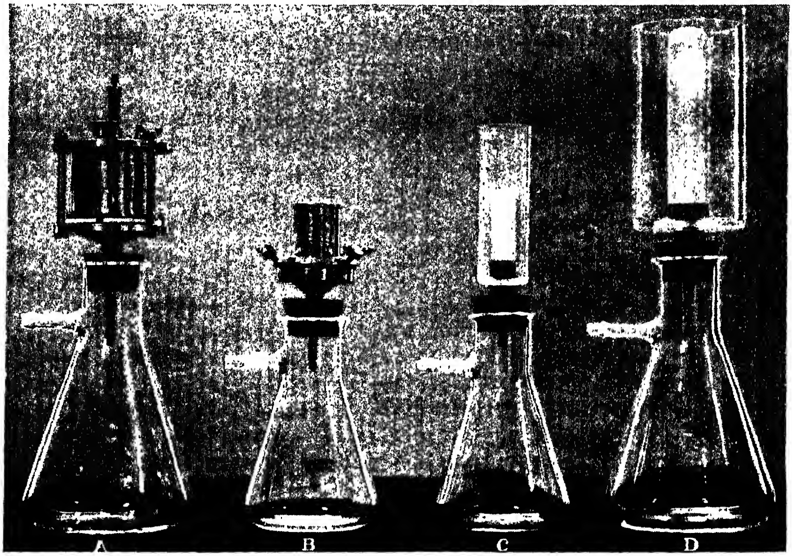


Fig. 2.—Some of the common filters used to remove bacteria from fluids. *A*, Seitz-Werke filter designed to operate with external pressure; *B*, small Seitz-Werke filter, suction model; *C* and *D*, Berkefeld filter candles mounted in glass mantles and set up for use.

unglazed porcelain. Occasionally filters for special purposes may be constructed of plaster of paris and similar substances. The fluid to be filtered is forced through these cylinders, commonly called "filter candles," by air pressure. An air pressure may be applied directly to the liquid as it is passing through the filter (positive pressure), or the receiving flask may be partially evacuated (negative pressure). In either case the result is the same; a differential pressure is obtained. This differential need not be great; one of 15 to 20 cm. of mercury is usually ample.

These filter candles may be obtained in varying degrees of fineness. The Berkefeld filter, a commonly used infusorial earth filter, is available in coarse, medium and fine porosities (designated as V or *viel*, N or *normal*

and W or *wenig*). The V filter will permit some bacteria to pass but is useful for preliminary filtrations to remove the majority of the bacteria from grossly contaminated fluids, while the N will usually remove all the cells and the W is even finer. The Chamberland filter, made of unglazed porcelain, is similarly graded, and the various porosities are designated as L 1, L 2, L 3, etc. The L 3 is roughly equivalent to the Berkefeld N. Minute defects in these candles sometimes occur, and filtrates must always be tested for sterility by the inoculation of a small amount into culture media.

In recent years the Seitz-Werke filter has come into use. This filter, which differs radically in construction from the filter candles, is made entirely of metal. The filter element is an asbestos pad which may be clamped into place. Each pad is used but once, and the problem of cleaning inherent in the earthen candles is eliminated.

The entire filtration apparatus must be assembled and sterilized before use. It is often convenient to sterilize an additional filter flask containing a cotton plug, for, after a filtration is complete, the filter may be removed and the sterile cotton plug inserted in its place. Bacterial fluids should be protected from light and kept in the ice-box after filtration. Candles may be cleaned by scrubbing with a stiff brush and by filtering a 10 per cent solution of potassium permanganate followed by 10 per cent oxalic acid. When this is done great care must be exercised to wash all the oxalic acid out of the candle by filtration of considerable quantities of clean water.

The sintered glass filter has come into quite general use in recent years and has many advantages. The sintered glass plate is prepared by grinding the glass, placing in a mold and heating just to the melting point, the glass particles adhering together to form a porous plate which may be sealed into any type of glass funnel to give an all-glass filter. The porosity depends upon the fineness to which the glass is ground. Bacteria-proof filters of this type were first made in Germany of Jena glass and recently have been made in this country of pyrex glass. They are cleaned with warm sulfuric acid containing about 1 per cent sodium nitrate; dichromate may not be used because it is adsorbed on to the sintered glass.

The Preparation of Culture Media.—The nutritive requirements of bacteria vary greatly, but those of most organisms are relatively simple and are adequately satisfied by meat infusions to which partially digested protein material, known under the chemically indefinite term peptone, is added. Although the great majority of bacteriological media are more or less complicated variations on this theme, the so-called "synthetic" media, or those of definite chemical composition, are often useful. In such solutions the source of carbon and energy is supplied in the form of *d*-glucose, lactate or similar compounds, and the nitrogen as an ammonium salt or a simple organic compound such as asparagin, the amide of aspartic acid. Ordinarily a buffer in the form of a phosphate is included, together with small amounts of inorganic compounds of iron, magnesium, etc. that may be appropriate. The medium of Uschinsky, of interest historically rather than because of any innate virtue, is representative of the class. It has the following composition:

water	1000 cc.
asparagin	4 gm.
ammonium lactate	6 gm.
Na_2HPO_4	2 gm.
NaCl	5 gm.

Such solutions are used for growing fungi such as yeasts and molds and for studies on bacterial nutrition.

Nutrient Broth.—Most bacteria, however, require peptones in combination with the extractives or water soluble constituents of lean meat. The culture medium most often used is nutrient broth—a medium in which the extractives are used in the form of "meat extract," a brown, sticky material resulting from the concentration of an aqueous extract of lean meat. Sodium chloride may or may not be added. The ingredients are weighed out in the following amounts:

peptone	10 gm.
meat extract	3.5 gm.
NaCl	5 gm.

and dissolved in 1000 cc. of water by heating. When solution is complete, the reaction is adjusted.

In recent years it has been shown by a number of workers including Mueller[1] and Gladstone and Fildes[2] that peptone and even meat extract may be replaced by appropriately prepared acid and enzymatic hydrolysates of casein.

Bacteria, like other living organisms, are relatively sensitive to the degree of acidity or alkalinity of their environment. Acidity is measured most conveniently in terms of the logarithm of the reciprocal of the hydrogen ion concentration, designated by the symbol *p*H. Neutrality is taken to be the reaction of pure water—water dissociating, of course, to hydrogen and hydroxyl ions, and in pure water the concentrations of the two are equal. The concentration of hydrogen ions in pure water is 1/10,000,000 mols per liter or 10^{-7} mols per liter. The reciprocal is 10,000,000 or 10^7 and the logarithm of the reciprocal 7, therefore *p*H = 7 at neutrality. Owing to the fact that *p*H is the logarithm of a *reciprocal,* it bears an inverse relation to acidity—the more acid a solution, the lower the *p*H. A solution whose *p*H = 1 is N/10, one whose *p*H = 2 is N/100.

Bacteria exhibit a somewhat greater-tolerance to variations in *p*H than other more delicate organisms, as a group thriving in *p*H's varying from 5 to 9. Although these organisms differ in their optimum *p*H's—the lactobacilli, for example, growing well at *p*H 5.0 while the cholera vibrio grows only in more alkaline solutions having a *p*H of approximately 8.0—most of them will grow well at *p*H 6.8–7.0. The reaction of a nutrient medium is, therefore, adjusted to approximately this point.

The most accurate measure of *p*H is electrometric,[3] using a hydrogen,

[1] *Cf.* Mueller and Johnson: Jour. Immunol., 1941, *40:*33.

[2] Gladstone and Fildes: Brit. Jour. Exp. Path., 1940, *21:*161.

[3] For details see Clark: *The Determination of Hydrogen Ions.* Williams and Wilkins. Baltimore. 1928.

quinhydrone or glass electrode, but *p*H also may be measured with a considerable degree of accuracy by means of appropriate dyes (the familiar indicators of analytical chemistry). Certain dyes have been found which change color at various points on the *p*H scale, bromthymol blue, for example, being yellow in acid solution and blue in alkaline solution and exhibiting color changes from yellow through a series of greens to blue within a *p*H range of 6.0 to 7.6. A series of standard buffer solutions of known *p*H may be prepared and a small quantity of dye added to each. The result is a series of solutions which exhibit the colors of the dye throughout its effective *p*H range and with which unknowns, to which the same dye has been added, may be compared. The accuracy is usually ± 0.1 unit of the *p*H scale.

The reaction of bacteriological media may be adjusted by trial or titrated in the following manner. Place 5 cc. of the medium in 20 cc. of neutral distilled water and add 10 drops of indicator. From a burette add N/20 sodium hydroxide (these nutrient media are usually too acid when prepared) until the color matches a solution prepared in the same way from standard buffer solution having the desired *p*H. The number of cubic centimeters of N/20 alkali used, multiplied by 10, gives the quantity of normal sodium hydroxide necessary to adjust 1 liter of medium. The reaction should be checked after final adjustment.

INDICATORS

Common Name	Concentration, Per Cent	Range, *p*H
Thymol blue.....	0.04	1.2–2.8
Bromphenol blue.	0.04	3.0–4.6
Bromcresol green.	0.04	4.0–5.5
Methyl red......	0.02	4.4–6.0
Propyl red.......	0.02	4.8–6.4
Bromcresol purple	0.04	5.2–6.8
Bromthymol blue.	0.04	6.0–7.6
Phenol red.......	0.02	6.8–8.4
Cresol red.......	0.02	7.2–8.8
Thymol blue.....	0.04	8.0–9.6
Cresol phthalein..	0.02	8.2–9.8

After adjustment the broth is heated in a flask for ten to fifteen minutes, allowed to cool again to bring down any precipitate that may have formed, and its final reaction checked; then it is filtered through paper, placed in flasks or tubes and sterilized. The *p*H usually drops slightly during sterilization.

Sugar Broths.—A series of media extremely useful in the differentiation of bacteria may be prepared from this nutrient broth base. One of the most useful groups is that of the sugar broths—nutrient broth to which has been added a sugar in a concentration of 0.5 to 1.0 per cent. The most commonly used sugars are dextrose, lactose and sucrose, and the

ability to ferment one or more of these is one of the most convenient, as well as one of the most informative, of the biochemical reactions. Other sugars, not only hexoses but pentoses, trioses and other polyhydric alcohols, may be and are used in the finer differentiation of some bacterial species. Sugars that may be partially decomposed by the higher temperatures of the autoclave may be sterilized separately by filtration and added aseptically to the sterile broth.

It is generally assumed that bacteria that will ferment a given sugar will ferment it to organic acids, lactic acid very often admixed with volatile acids, *e.g.*, formic, acetic, propionic, butyric acids. The end products of bacterial fermentation of sugars may include compounds other than organic acids, such as ethyl and butyl alcohols, acetone, glycols and the like, together with hydrogen and carbon dioxide. However, the usual appearance of organic acids when a sugar has been fermented has been taken,

STANDARD PHOSPHATE BUFFER SOLUTIONS

*p*H	cc. M/15 KH_2PO_4	cc. M/15 Na_2HPO_4
5.3	19.5	0.5
5.5	19.2	0.8
5.8	18.4	1.6
6.0	17.5	2.5
6.2	16.1	3.9
6.4	14.5	5.5
6.6	12.3	7.7
6.8	10.0	10.0
7.0	7.8	12.2
7.2	5.7	14.3
7.4	4.0	16.0
7.6	2.6	17.4
7.8	1.6	18.4
8.0	1.0	19.0
8.3	0.5	19.5

together with the formation of gases, as a criterion of fermentation. The accumulation of such acids during the course of fermentation is made readily apparent by the incorporation of a suitable indicator dye in the sugar broth. Either bromcresol purple or bromthymol blue may be used for this purpose, for neither is toxic or inhibitory to bacterial growth. A tube of nutrient broth containing a sugar and indicator is, of course, purple or a bluish green, the color depending upon what dye is added. After inoculation with a pure culture of bacteria that will ferment the sugar, and incubation for twenty-four hours or more, the medium will be found to be turbid with growth and yellow in color as a result of the acid reaction produced. Conversely, if the sugar is not fermented the broth will be turbid but the color will remain unchanged.

Gases, hydrogen and carbon dioxide, may or may not result from the fermentation of a given sugar by a particular bacterium. It may be, and usually is, worth while to know whether or not an organism will produce

gas from a sugar and, consequently, various methods have been used to collect some of the gas if it is formed. The simplest of these devices is the Durham fermentation tube, an ordinary test tube in which a small vial is inverted. The vial, of course, becomes completely filled with medium during sterilization. If gas is formed during the fermentation, some of it will collect in the vial and displace a portion of the medium. Such a method can give only qualitative results. Crudely quantitative results may be obtained by the use of special tubes with closed arms. One advantage of carrying out fermentation tests in such tubes is that an approximation of the gas ratio may be made. Some bacteria characteristically form equal parts of carbon dioxide and hydrogen, while others form two parts of hydrogen to one of carbon dioxide. If, after fermentation is complete, the volume of gas in the closed arm is read, and then alkali is added to the culture and thoroughly mixed with it, it will be found that the gas volume has diminished. It may be assumed that the portion which disappears is carbon dioxide which has been absorbed by the alkali and that the part which remains is hydrogen. Such results are obviously only rough approximations. Precise measurements of carbon dioxide and hydrogen evolved are made by other means.

Media for Other Biochemical Tests.—The determination of some other biochemical properties of bacteria is a part of the routine examination and differentiation of these organisms. The most common of these are the ability to reduce nitrates, to produce indol, and to hydrolyze gelatin and starch. The last two will be discussed under the semisolid media.

Nitrate Reduction.—The reducing ability of an organism may be manifested in a number of ways. These reactions, which are somewhat more complex than might be thought, are discussed briefly in a later chapter (p. 85). The reduction of indicator dyes is not uncommon. Many dyes lose their color when reduced, and it is sometimes a source of concern to the beginning student to find that because his indicator has apparently disappeared he is unable to tell whether or not a given sugar has been fermented.

The ability to reduce nitrates is usually specifically tested for by the inoculation of nitrate broth (containing 0.02 per cent nitrite-free potassium nitrate and peptone but no meat extract) and, after four days' incubation, testing for the presence of nitrite. This is readily done by means of the nitroso reaction: the addition of 2 cc. each of a sulfanilic acid reagent and α-naphthylamine reagent[4] to 3 to 5 cc. of culture. The development of a rose color indicates the presence of nitrite. The test is extremely sensitive, and a control test must be carried out on a tube of the uninoculated nitrate broth; otherwise one may be deceived by false positive reactions resulting from contamination of the original potassium nitrate with nitrite.

Indol.—Indol is formed from the amino acid tryptophane by some bacteria and apparently not by others; the presence or absence of this

[4] The sulfanilic acid reagent is prepared by dissolving 8 gm. of sulfanilic acid in 1000 cc. of 5N acetic acid. The α-naphthylamine reagent (α-amido-naphthylene acetate) is prepared by dissolving 5 gm. of α-naphthylamine in 1000 cc. of 5N acetic acid and clearing by filtration through absorbent cotton.

substance is, therefore, of some significance in the separation of groups or species of bacteria from one another. The organisms may be grown in peptone water (1 per cent peptone and 0.5 per cent sodium chloride and no meat extract) and, after four days' incubation, the cultures tested for the presence of indol by the judicious addition of Ehrlich's reagent[5] in such a manner that it forms a layer on the surface of the medium. The development of a red color at the interface indicates the presence of indol. The specificity may be further enhanced by shaking the mixture with amyl alcohol, for the compound formed is soluble in this solvent. Whether or not a strongly positive reaction is obtained depends to a considerable degree on the amount of tryptophane present in the culture medium. Proteins vary considerably in their tryptophane content, and, in consequence, peptones prepared from different proteins will also vary. Since tryptophane is destroyed by acid hydrolysis, the peptone must be prepared by enzymatic digestion. Tryptophane may, of course, be added to the peptone water during its preparation, thereby ensuring its presence in ample quantities.

Milk.—Perhaps the most useful single medium employed in routine bacteriological work is milk with an indicator added. The indicator is usually litmus, but bromcresol purple has been found satisfactory also. Milk contains both lactose and casein, and bacterial growth in this medium may result in any of a series of changes, all of which are highly informative.

If lactose is fermented, the indicator will show the acid reaction resulting; and if considerable quantities of acid accumulate, the casein will be precipitated with the formation of an *acid curd.* If lactose is not fermented, there may be a slight transient acidity owing to the fermentation of small quantities of dextrose present, followed by an increasingly alkaline reaction resulting from the accumulation of amines and other basic compounds. The casein may or may not be precipitated as a *rennin curd.* If such precipitation does occur, proteolysis, or *peptonization,* as it is called, may proceed to such a point that the milk is no longer opaque but becomes clear and a light brown in color. The indicator may be reduced under either acid or alkaline conditions. These types of change may be summarized briefly:

(1) The development of an alkaline reaction, usually after three to four days' incubation.
(2) The development of an alkaline reaction with precipitation of the casein as a rennet curd, with or without reduction of the indicator.
(3) The development of alkalinity and rennet curd precipitation, followed by peptonization of the curd; the indicator is usually reduced by the time digestion is apparent.
(4) Acid formation, usually in twenty-four hours, with or without reduction of the indicator.
(5) Acid formation and precipitation of the casein, usually with reduction of the indicator.

The culture of bacteria in milk, then, may yield a great deal of information regarding their physiological capabilities.

[5] Ehrlich's reagent consists of 4 gm. of *p*-dimethylamidobenzaldehyde dissolved in a mixture of 380 cc. of ethyl alcohol and 80 cc. of concentrated hydrochloric acid.

The medium itself is skimmed milk to which the indicator has been added in the amount of 5 cc. of a 0.25 per cent alcoholic solution to each liter of medium. Skim milk powder is most convenient to use in the laboratory. One hundred and fifty grams of the powder is rubbed up in a mortar with a small quantity of water and then diluted to 1 liter. Indicator is added in the same proportion. Medium prepared from powdered milk is autoclaved, but that prepared with fresh milk should be sterilized by the intermittent method in the Arnold.

Infusion Media.—Nutrient broth, while a satisfactory medium for many bacteria, does not provide the more fastidious organisms with all the nutriment necessary to their growth. This deficiency may be made up, to a considerable extent, by the use of infusions of fresh meat rather than the prepared meat extract. Beef or veal is ordinarily used. The latter is thought by some to provide a somewhat richer medium than beef. In either case the procedure of preparation is the same, the desired result being an aqueous solution of the water-soluble constituents of the tissue.

For the preparation of 1 liter of beef infusion broth 500 gm. (about 1 pound) of chopped lean beef are placed in 1 liter of distilled water and placed in the ice-box over night. The infusion is boiled for half an hour the next day to precipitate coagulable protein and filtered through paper or cheesecloth. Ten grams of peptone and 5 gm. of sodium chloride are added, the volume made up to 1 liter and the reaction adjusted. After filtration, the medium is sterilized in appropriate containers.

Such media may be further enriched in a variety of ways. The addition of small amounts of glucose and phosphate buffer, for example, greatly facilitates the growth of streptococci. A variety of special infusion media have been proposed for special purposes. Not only infusions of other animal tissues, such as liver, but also certain vegetable infusions have proven useful. For a discussion of the last see Brewer.[6] The student will find these and other media discussed at greater length elsewhere.[7] Such infusion media may be used as a base for media designed for particular purposes just as nutrient broth is used. For example, sugar media consisting of beef infusion broth plus the desired sugars are used in the study of the fermentation reactions of the more fastidious bacteria.

The Semisolid Media.—The semisolid media consist of a liquid nutrient medium, such as nutrient broth or infusion broth, solidified by the addition of gelatin or agar in quantities of 10 to 12 per cent and 1.5 per cent respectively. Gelatin is, of course, liquid at temperatures above 30° C., but an agar gel has the peculiar, but bacteriologically advantageous, properties of not liquefying until it reaches about 100° C. and, once liquefied, of not solidifying again until the solution has cooled to about 40° C. The term semisolid derives from the fact that such media may be either liquid or solid in the form of gels.

By far the most important and extensive use to which semisolid media are put is in the isolation of bacteria in pure culture. Gelatin media are

[6] Brewer: Jour. Bact., 1943, *46:*395.

[7] Levine and Schoenlein: *A Compilation of Culture Media for The Cultivation of Microorganisms.* Williams & Wilkins Company. Baltimore. 1930.

not so satisfactory for this purpose as are agar media because the gelatin is liquid at the temperatures at which most pathogenic bacteria grow and because it is itself digested and liquefied by some bacteria. In spite of these defects, however, gelatin has been and is useful in water bacteriology. It was used as a solidifying agent by Koch before agar was introduced by one of his technicians, Frau Hesse.[8] Agar, a polysaccharide obtained from certain species of seaweed, is not attacked by bacteria.[9]

The semisolid media may be enriched and their compositions varied within wide limits to conform with the requirements of particular problems. One of the commonest means of enrichment is that of the addition of 10 per cent defibrinated blood (usually sheep). Not only will a blood agar prepared from an infusion base support the growth of some of the most fastidious bacteria but, because of the blood present, the ability of certain organisms to elaborate hemolysins, substances which digest the blood, is made evident by the appearance of green or clear zones about their colonies.

The Hydrolysis of Gelatin, Starch and Fat.—The ability to digest protein is possessed to a marked degree by some, the so-called proteolytic, bacteria and not by others. Gelatin, although not a native protein (it is derived from collagen by hydrolysis), is useful in the detection of this property since when decomposed it loses its ability to form a gel. Gelatin liquefaction is demonstrated by allowing nutrient gelatin, nutrient broth solidified by the addition of gelatin, to gel in test tubes and inoculating by stabbing the inoculum deep into the medium with the inoculating wire. Not only is the ability to digest gelatin shown by the appearance of a zone of liquefaction about the stab, but the shape of this zone is often characteristic of certain bacterial species. Gelatin cultures must, of course, be kept at temperatures below the point at which they liquefy, usually at 20° to 22° C. The problem of gelatin liquefaction by bacteria which will not grow at these lower temperatures may be solved by incubating the cultures at 37° C., at which the gelatin is liquid, and then, after a suitable period of incubation, cooling the tubes in cold water. If the gelatin resolidifies, presumably no liquefaction has taken place; if it does not, it may be assumed that some of it has been digested. This method is not particularly reliable, and other methods such as the titration of amino acids liberated by digestion (formol titration) have been suggested. None of these, however, is entirely satisfactory for routine laboratory work. Proteolysis may also be detected in cultures on coagulated serum medium; lacunae of liquefaction form about the colonies.

The hydrolysis of starch may be demonstrated by growth on nutrient agar containing 1 per cent starch. The medium is usually melted and poured into a sterile Petri dish. The solid medium is inoculated by a streak across the center of the plate. After incubation Lugol's iodine solution is poured on the plate. The presence of starch is indicated by the development of a blue color. If the starch has been hydrolyzed by the bacteria, a clear, colorless zone will surround the streak of growth.

[8] *Cf.* Hitchens and Leikind: Jour. Bact., 1938, *35*:21.

[9] Rare forms have been reported which hydrolyze agar.

The hydrolysis of fats may be observed by incorporating 1 per cent tributyrin in the agar medium; hydrolysis is indicated by the development of a zone of clearing around the colony.[10] Basic dyes, especially Nile blue, may be used for the detection of lipolysis in media containing an emulsified oil such as cottonseed oil.[11] Starr[12] recommends the use of spirit blue.

Lead Acetate Agar.—A further differential characteristic observed among the bacteria is the production of hydrogen sulfide (presumably from sulfur-containing amino acids present in the peptone). The formation of hydrogen sulfide is readily detected by the use of nutrient agar containing 0.05 per cent basic lead acetate. A tube of the solid medium is inoculated by a deep stab, and the production of hydrogen sulfide is indicated by the presence of a black precipitate of lead sulfide along the line of growth.

Selective and Differential Media.—As pointed out above, bacteriological media may be modified in many ways for special purposes. By the inclusion of appropriate substances, media may be rendered selective—*i.e.*, they may favor the growth of some kinds of organisms and suppress that of others. Differential media may be prepared which disclose or exaggerate characteristics of the particular organism of interest, thereby considerably facilitating its isolation. If, for example, dextrose is added to nutrient agar and the *p*H lowered to 5.0 to 5.5. by the addition of lactic acid, the resulting medium is too acid for most bacteria (except the so-called aciduric forms) but yeasts and molds grow well on it. In the study of these fungi one may, therefore, eliminate much extraneous bacterial contamination by the use of such a medium.

The addition of indicators to solid sugar media is often a desirable procedure. When mixtures of bacteria are inoculated on to litmus lactose agar plates, for example, the colonies of the lactose-fermenting bacteria will be red because of the production of acid and consequent change in color of the indicator, in contrast to the blue colonies of the other organisms. Endo's medium, one commonly used in the study of bacteria of the colon-typhoid group, contains lactose, and Schiff's reagent, basic fuchsin decolorized by sodium sulfite. Aldehydes restore the color to this reagent, and it is used by the biochemist in qualitative tests for these compounds. Lactose-fermenting bacteria develop red colonies on Endo's medium, the color a result of the presence of aldehydes formed as intermediates in the breakdown of the sugar (p. 102). Further, colonies of the colon bacillus on this medium acquire a metallic sheen which they do not show on nutrient agar.

The addition of dyes which inhibit the growth of some bacteria more than that of others is common also (p. 68). A medium containing brilliant green is extremely useful in the isolation of the typhoid bacillus. The dye markedly inhibits the growth of other organisms, including the colon and dysentery bacilli, but, in appropriate concentrations, allows the growth

[10] Anderson: Jour. Bact., 1934, *27*:69.
[11] See the discussion by Knaysi: Jour. Bact., 1941, *42*:587.
[12] Starr: Science, 1941, *93*:333.

of the typhoid bacillus. The utility of such a medium in the examination of fecal specimens is obvious. Another medium containing eosin and methylene blue is useful in the isolation of the dysentery bacilli and similar organisms, partly because of selective inhibitory activity and partly because colony differences are considerably accentuated.

Some of the most selective media devised are those used for growing the autotrophic bacteria. These organisms, deriving their energy from the oxidation of ammonia and nitrite, are poisoned by organic matter, even agar. Liquid media containing ammonia as a source of nitrogen and energy and carbonate as a source of carbon will obviously support the growth of those organisms oxidizing ammonia to nitrite, and no others. The substitution of a nitrite for the ammonium salt renders the medium suitable only for those bacteria which oxidize nitrite to nitrate. Such media cannot be solidified by agar or gelatin, but instead a silica gel is used.

Methods of Obtaining Pure Cultures.—When fluid culture media are inoculated with substances such as soil, water or excreta, many kinds of organisms develop simultaneously side by side, and a heterogeneous mixture, or "mixed culture," of bacteria results. This fact greatly hampered the earlier workers, and it was not until methods for obtaining "pure cultures," or cultures containing only one kind of bacteria, were devised that experimental bacteriology was placed upon a firm foundation.

Any technical procedure for obtaining such pure cultures is dependent upon the isolation of a single viable bacterium which is allowed to multiply in a suitable culture medium. Bacteriologists first attempted such isolation by simple dilution, *i.e.*, dilution of a contaminated fluid with sterile water to a point at which a single cubic centimeter contained, theoretically, only one bacterium. Portions of these highly diluted liquids were cultured in hope that in some cases at least only a single bacterium was present. This method was, however, inherently unsatisfactory in that the experimenter had no assurance that his "pure culture" consisted of the descendants of only a single bacterium. The first reliable method of isolation of pure cultures was devised by Koch in 1881. This method, the use of semisolid media, has proved so satisfactory that it has been employed to the present day with only minor modifications. If nutrient gelatin or agar is inoculated while fluid (for example, at 42° C.) and is then solidified and kept under favorable temperature conditions, many of the living bacteria that have been introduced are able to multiply. Since the bacteria cannot move about freely, but are fixed in the stiffened medium, the progeny of each organism form distinct masses or colonies. These colonies consist of many millions of bacteria and are readily visible to the naked eye or by means of a low power hand lens. If the colonies are not closely crowded, a pure culture may be obtained by touching a colony with the tip of a sterile needle and inoculating tubes of fresh culture media. In order to secure a large surface upon which the colonies shall be spread out and made easily accessible, the gelatin or agar after inoculation is poured while still fluid into sterilized flat shallow dishes (Petri dishes) fitted with glass covers.

It will be clear that a given colony may arise from two or more parent cells if these remain attached or close together in the agar medium, and the colony will not be a pure culture in the event that the juxtaposed cells are of different species. This possibility has been investigated by McNew[13] using the plant pathogen *Phytomonas stewarti*. He found that 98 per cent or more of the colonies arose from single cells, hence the probability of a mixed culture in a single colony is 0.02 or less. Successive plating or picking a number of colonies makes a pure culture a practical certainty. It is likely, however, that this figure differs for different bacteria, *i.e.*, staphylococci are more likely to remain attached than micrococci or bacilli.

Within recent years methods have been devised whereby a single bacterium may be picked out of a bacterial suspension by mechanical means and cultured in nutrient media. For such work an elaborate apparatus known as a micromanipulator is necessary, and the operator picks out a single bacterium with a fine capillary pipette. The entire process is observed through the high power oil immersion lens. A high degree of technical skill is essential for such work and this procedure is used only when the greatest precautions must be taken to ensure pure cultures.

It is self-evident that the pure culture so obtained is not only homogeneous with respect to kind or species of bacteria, but since the microorganisms are descendents of a single parent cell, they constitute what the zoologist or protozoologist terms a *clone*.

Technique of Making Plate Cultures.—Three tubes of agar (1, 2, 3), melted at 100° C., are placed in a water bath at 42° C., a temperature that is just above the solidifying point of agar and is not injurious to bacteria. Tube 1 is inoculated with a loopful of the material to be plated.[14] The cotton plug is then replaced and the contents of the tube are mixed by carefully tilting back and forth and rotating the tube on its long axis. From this tube two loopfuls of agar are transferred to tube 2, and after mixing, two more loopfuls carried from tube 2 to tube 3. The contents of the several tubes are then poured into Petri dishes. As soon as the cotton plug is removed, the mouth of each tube should be passed through the flame and inserted under the edge of the lifted Petri dish cover, and the agar quickly poured out. The covered Petri dish may then be tipped cautiously back and forth to distribute the agar evenly before it solidifies. Agar plates placed in the incubator after solidification should be inverted in order to avoid spreading of the growth through condensation of moisture on the surface of the medium. Even if there are a great many bacteria in the original material, the plate from tube 3 will probably contain the organisms in small enough numbers to develop well-isolated colonies. On the other hand, if there are very few bacteria in the material inoculated, plate 1 will probably present more satisfactory conditions. Gelatin plates are made in the same manner as agar except that gelatin may be cooled as low as 25° C. without solidifying.

[13] McNew: Phytopathology, 1938, *28*:387.

[14] It is often desirable to make first a suspension in salt solution, as, for example, in dealing with material like pus.

Under exceptional conditions, such as work in the field, when Petri dishes are not available, so-called "roll tubes" may be made. The tubes containing the liquid agar medium are tilted until the agar almost reaches the cotton plug. The tubes are then rotated in this position against a block of ice, and when the process is complete the test tube is coated on the inside with a thin layer of solid medium. In this way a considerable surface is obtained and, after incubation, colonies are readily picked.

Quantitative Dilution.—It is sometimes of advantage before plating to make accurate dilutions of highly polluted fluids, such as sewage, in order to get colonies few enough in number to be well isolated. If there is reason to suppose that the number of bacteria is 200 per cc. or more, 1 cc. of the sample is mixed with 9 cc. of sterile water. If a higher dilution is required, proceed in a similar manner.

(A)	To dilute	1: 10	use 1 cc. of sample	to 9	cc. of sterile water.
(B)	"	1: 100	"	" 99	
(C)	"	1: 1,000	(A)	" 99	
(D)	"	1: 10,000	(B)	" 99	
(E)	"	1: 100,000	(C)	" 99	
(F)	"	1:1,000,000	(D)	" 99	

One cubic centimeter of each dilution is placed in a sterile Petri dish and 6 to 8 cc. of liquid cooled agar medium are added. The two are thoroughly mixed by tilting the Petri dish back and forth and then allowed to solidify. After incubation the total number of colonies on the plates containing 50 to 200 colonies is counted and the total number is multiplied by the dilution to give the number of organisms present in the original sample. Such bacterial counts are relatively inaccurate, errors of 10 to 15 per cent being common in even the most careful work, and must be regarded only as useful approximations.

Streak Plates.—Not infrequently media used for the isolation of the more fastidious bacteria are such that it is technically difficult to make the usual pour plates. The most common of these media are those which contain fresh blood. Such media are ordinarily prepared in quantity and sterile plates poured. These may be stored in the ice-box until used. Dilution and consequent isolation of bacteria may be accomplished with these media by a process known as streaking. A sterile wire loop is dipped into the bacterial suspension and rubbed back and forth on the surface of the solid medium, making successive streaks as close together as possible. After incubation it will be found that at some point sufficient numbers of bacteria were rubbed off the loop to allow the development of individual colonies in the path of the streak. These may be picked and transferred to fresh media. Quantitative dilutions may be prepared in sterile broth or saline and 0.5 cc. of each dilution pipetted on to the surface of the medium and spread evenly by appropriate tilting.

The Separation of Bacterial Species by Other Means.—*Heat.*—Spore-forming organisms are sometimes separated from other bacteria by heating mixed cultures containing spores to 80° C. for fifteen minutes.

This procedure kills off any vegetative forms that may be present, but leaves the heat-resistant spores able to develop if placed under favorable conditions. This method is widely used in the isolation of the sporulating anaerobes. A further separation of different varieties of spore-forming organisms must then be effected by plating or animal inoculation.

Animal Inoculation.—Certain pathogenic bacteria that often occur in the animal body mixed with other species, as is the case, for example, with *Mycobacterium tuberculosis* or *Clostridium tetani*, are sometimes obtained free from other bacteria by inoculating an animal with the material containing the mixture of organisms. After allowing time for the bacteria to develop, the animal is killed, and tubes of suitable media are inoculated from the characteristic lesions; in such cases the specific bacillus will often be found in pure culture in the tissues.

Methods of Growing Anaerobes.—The oxygen of the atmosphere is apparently toxic to some kinds of bacteria, and these organisms are unable to grow in its presence (p. 90). Various methods have been devised for the cultivation of these organisms, all of which center about the elimination of oxygen from the gaseous environment of the cultures.

One of the simplest methods is that of the "shake culture," in which a semisolid medium is boiled to drive out dissolved oxygen, cooled quickly to 42° C. and inoculated. Colonies of anaerobes develop deep in the agar medium. Liquid media may be similarly boiled and cooled quickly just before inoculation, and sometimes sterile oil or a mixture of petrolatum and paraffin layered on the surface to prevent diffusion of oxygen into the liquid. Such seals, however, are not particularly effective. The method is satisfactory, especially when rich media and heavy inoculations are used. Obligate anaerobes may often be kept in stock culture as mixed cultures with an aerobic organism, the latter using up the oxygen in the medium in its metabolic processes, thereby creating anaerobic conditions which allow the development of the anaerobe. The most important obligate anaerobes form spores, and if the aerobic organism used does not, separation of the anaerobe from the mixed culture by heat is not difficult.

The removal of oxygen by combustion has been a widely used method. The inoculated culture media are placed in a container—an anaerobic jar or vacuum desiccator—and oxygen is removed from the air within the container. Necessarily, the joint between lid and container must be gas tight. A ground glass joint lubricated with stopcock grease is generally satisfactory. The combustion may be carried out by saturating a piece of filter paper with ethyl alcohol, igniting it within the container, and quickly replacing the lid. Theoretically the oxygen is removed by the combustion of the alcohol, but practically the method is not always successful. Similarly, oxygen may be removed by burning phosphorus within the jar, the phosphorus pentoxide formed being removed by solution in a small amount of water in the bottom of the jar. Still another method has been used in which oxygen is removed by combination with hydrogen in the presence of heated platinized asbestos. The asbestos is heated by an electric coil and hydrogen run in judiciously. Oxygen is efficiently removed, but the hazard of explosion is not negligible.

A method which has been generally used because of its uniform success is that of absorption of oxygen by a mixture of pyrogallic acid and alkali. Dry pyrogallic acid (10 gm. per liter of air space) is placed in the bottom of the jar and 150 cc. of 1 per cent sodium hydroxide solution poured in and the cover replaced. An excess of alkali is to be avoided, for it will also absorb carbon dioxide, traces of which appear to be necessary to the growth of some bacteria (p. 125). Dack has elaborated a variation on this method which is highly successful. After the pyrogallic acid and alkali (sodium carbonate in this case) have been mixed, he evacuates the jar and replaces the air with carbon dioxide. This is in turn partially removed by evacuation, leaving 25 to 30 cm. Hg carbon dioxide pressure, and the vacuum desiccator sealed by closing the stopcock. This method has the great advantage that breakdown of anaerobic conditions by leakage of air during incubation is indicated by browning of the solution in the bottom of the jar. The pyrogallic acid method may also be used directly in culture tubes, a stopper of absorbent cotton being pushed down to leave an inch space at the top of the tube, pyrogallic acid and sodium hydroxide solution placed in this space, the cotton saturated, and the tube closed at once with a tight-fitting rubber stopper and incubated upside down.

Rosenthal[15] has suggested the use of sulfuric acid and chromium metal powder for the removal of oxygen from anaerobic containers, the hydrogen evolved driving out the air in the jar and the chromous sulfate absorbing oxygen in the presence of sulfuric acid some forty times faster than alkaline pyrogallate.

Single Petri dish cultures may be incubated anaerobically by the use of the Spray dish. This dish is constructed with a lip around the top into which the lower half of a Petri dish may be set and sealed in with paraffin. The bottom is divided into two parts by a ridge of glass extending across the center. Pyrogallic acid is placed in one half of the bottom and a solution of alkali in the other. After inoculation the lower half of a Petri dish is inverted over the Spray dish and sealed in with paraffin. When the pyrogallic acid and alkali are mixed by tilting, oxygen is absorbed and obligate anaerobes will grow on the surface of the agar medium.

An "anaerobic" Petri dish cover has recently been introduced by Brewer[16] which permits the anaerobic incubation of a single dish without the use of other apparatus.

Air can, of course, be replaced in an anaerobic jar by some inert gas such as hydrogen or nitrogen. The former is more often used, for tanked nitrogen contains small quantities of oxygen, sufficient to prevent growth of some of the obligate anaerobes. If some such replacement scheme is used, special anaerobic jars, like those designed by Novy, are more satisfactory than the ordinary vacuum desiccator.

Special liquid media in which anaerobic conditions are attained by the medium itself are often used for the cultivation of obligate anaerobes. The solutions are enriched by the addition of animal tissue; for example,

[15] Rosenthal: Jour. Bact., 1937, *34*:317.
[16] Brewer: Science, 1942, *95*:587.

a sterile piece of fresh tissue such as rabbit kidney may be added to the previously sterilized medium, or finely chopped beef heart may be added before sterilization and autoclaved in the medium. Dextrose is almost always included with the infusion base. Such media show strong oxygen

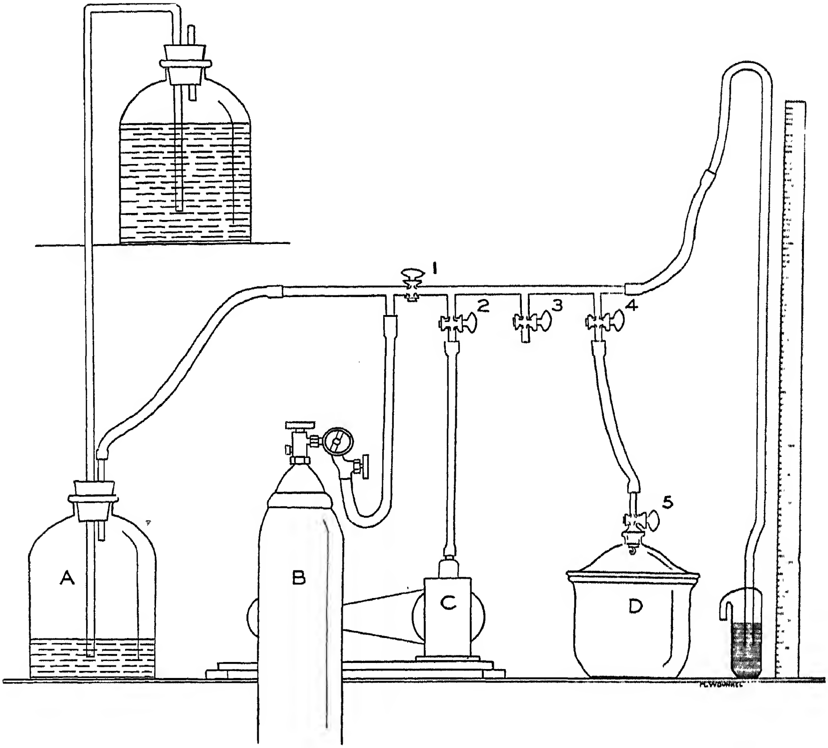


Fig. 3.—Dack's anaerobic apparatus. *A*, gas reservoir; *B*, carbon dioxide tank; *C*, vacuum pump; *D*, desiccator. Culture tubes and plates are placed in the desiccator, stopcocks 1 and 3 closed, and the system evacuated to *ca.* 8 to 10 cm. of mercury. Stopcock 2 is closed and stopcock 1 opened, allowing carbon dioxide from the gas reservoir to flow into the system until a pressure of 50 to 60 cm. has been reached. Stopcock 1 is closed and stopcock 2 opened and the system again evacuated to a pressure of *ca.* 20 cm. of mercury. Stopcock 5 is then closed and stopcock 3 opened and the desiccator removed and placed in the incubator. Gas reservoir *A* is refilled from the tank whenever necessary but carbon dioxide is not allowed to flow directly from the tank into the desiccator. Traces of oxygen remaining in the closed desiccator are removed by the pyrogallic acid-sodium carbonate mixture.

uptakes, either as a result of respiratory processes of fresh tissue or of the catalytic oxidation of reduced compounds (such as cysteine or reduced glutathione) present in the sterilized tissue. Oxygen is taken up more rapidly than it diffuses into the solutions and, in consequence, anaerobic conditions prevail.

Animal Inoculation.—An indispensable adjunct to the study of the pathogenic bacteria is the experimental animal. Animals are used not only for the study of the pathology of infectious disease and as an aid in the isolation of some bacteria in pure culture, but also for the experimental production of immune sera and studies on the various manifestations of immune phenomena. The maintenance of such infectious agents as the filterable viruses, which can not be cultivated on lifeless media, by animal passage is common also. The animals generally used are rabbits, guinea pigs, white mice and white rats, although in special cases others, such as rhesus monkeys, are necessary.

Experimental animals may be inoculated by a variety of routes, usually one or another being preferable under the particular circumstances. The common routes of inoculation are intradermal, subcutaneous, intramuscular, intraperitoneal and intravenous. Other routes such as intraocular, intracerebral, intrathecal, etc., may be desirable at times. In any case, inoculation is carried out with a syringe and hypodermic needle, the capacity of the syringe and size of the needle used depending upon the quantity of material to be injected and the route of inoculation.

The site of inoculation must be prepared by removal of the hair by shaving or a depilatory, followed by disinfection by swabbing with alcohol, tincture of iodine, etc. The syringe and needles have, of course, been previously sterilized by dry heat or by boiling. Material inoculated *intracutaneously* is inoculated into the skin. A fold of skin is pinched up and the needle inserted, lumen up, as superficially as possible. A raised white spot showing the pits of the hair follicles indicates a successful injection. Not more than 0.2 cc. may be inoculated by this route. *Subcutaneous* inoculation requires considerably less skill on the part of the operator. A fold of skin is pinched up as before and the needle inserted through the skin to its full length. The injected material forms a bleb or blister. The amount that may be injected by this route depends upon the size of the animal. In the *intramuscular* inoculation, the needle is inserted deep into the muscular tissue, usually the posterior muscles of the thigh or the lateral thoracic or abdominal muscles. *Intraperitoneal* inoculation is carried out as one would a subcutaneous injection on the abdomen except that after the needle penetrates the skin, it is held at right angles to the peritoneal wall and thrust through into the peritoneal cavity. *Intravenous* inoculation may be carried out in a variety of ways, the choice depending upon the size of the animal. Rabbits are conveniently inoculated in the marginal vein of the ear. The needle is inserted through the skin and into the vein from the side. If the needle is within the vein, blood will disappear in the vein from the point of the needle onward as the material is injected. Needless to say, the injection should take place in the direction of blood flow. Guinea pigs may be inoculated into the large superficial vein on the dorsal and inner side of the hind leg or into one of the external jugular veins. Both require anesthesia of the animal and a small incision, which may be later closed with a stitch or collodion. Rats may be inoculated in an external jugular vein and mice in one of the lateral veins of the tail.

Drawing Blood.—Blood may be taken from the rabbit from the marginal vein of the ear or directly from the heart. The appropriate area is shaved and swabbed with alcohol. If blood is to be taken from the ear, the marginal vein is cut across with a razor blade and the blood allowed to drop into a centrifuge tube or test tube. Flow may be hastened by preliminary application of a towel wrung out of hot water to induce hyperemia. When blood is to be taken directly from the heart, a 30 to 50 cc. syringe is used and the needle inserted between the ribs at the point of maximum pulsation and thrust into the heart, taking care to injure the pericardium as little as possible. Blood is most conveniently taken from smaller animals by this means.

Postmortem Examination.—The autopsy of experimental animals dead of infection is often highly desirable. Not only may cultures be taken but the gross and microscopic pathology are usually of significance. The animal is fastened down on a board, ventral surface up. It is good practice to have the board in a larger enameled tray to reduce to a minimum the danger of spreading infectious material. When cultures are to be taken, the animal must be opened with sterile instruments. Usually several pairs of scissors and forceps and two or three scalpels are necessary. The site of inoculation is examined for ulcerative or other changes, and the hair and skin are disinfected with cresol or lysol. The skin is incised from the pubis to the neck and cut at right angles at the ends of the incision. The two flaps may be laid back by separation of the cutaneous from the underlying muscular tissue with a scalpel. The condition of the subcutaneous tissue and of axillary and inguinal lymph glands may be noted. The peritoneal fluid may be cultured by searing a small area on the surface of the abdomen with a hot spatula and puncturing with a syringe and needle or a Pasteur pipette into which the fluid may be aspirated. The abdominal cavity is opened with fresh instruments and the flaps are laid back as above. The condition of the abdominal organs, such as spleen and liver, may be noted and cultures taken by first searing the surface and then either puncturing and aspirating fluid with a syringe and needle or a Pasteur pipette, or by removing a small piece of tissue and placing it in nutrient medium. Likewise, pieces of tissue may be removed and placed in fixing fluid for subsequent sectioning. Again with fresh instruments, the costal cartilages are cut to make a V-shaped incision and this flap is laid back over the head, exposing the thoracic cavity. Any gross pathology may be noted, cultures may be taken from heart's blood and elsewhere if desirable, and pieces of tissue removed for fixation and sectioning. The animal is disposed of by incineration.

It is obviously impossible to define postmortem procedure rigorously. The lymphatic system of guinea pigs dead of tuberculous infection will be of considerable interest. Those dying of diphtheritic toxemia or infection will show characteristic lesions of the adrenals. In some diseases the important postmortem findings may be in the brain and spinal cord. The routine of examination will be determined, therefore, by the particular disease under consideration or, if this is not known, by the symptoms exhibited by the animal before death.

A number of precautions must be observed in the autopsy of animals dead of infectious disease. If cultures are to be taken, fresh sterile instruments must be used at each stage. Instruments once used should be returned to the boiling water sterilizer or placed in a tray containing lysol or, if to be used again, laid down on the edge of the tray with all sharp edges and points inward. Extreme care must be taken to avoid disseminating infectious material. With certain highly contagious diseases such as tularemia, rubber gloves may be desirable. Boiling water sterilization (five minutes) of used instruments is sufficient except with the spore-forming organisms such as anthrax and tetanus, in which case the instruments should be allowed to stand in a solution of lysol or cresol.

The Microscopic Examination of Bacteria.—The microscopic examination of bacteria is an essential portion of studies on these organisms, for only by this means may a number of important characteristics be ascertained. The organisms are observed both in the living state and when killed and fixed in the form of a film on a glass slide.

Some bacteria, as a result of the possession of locomotive structures, exhibit motility, or the ability to move about independently (p. 42). Others, not having such appendages, are not able to do so. For observation of motility—which can, obviously, be seen only in living organisms—the hanging drop preparation is used. When the organisms are growing in a fluid medium, a drop of culture is placed upon a clean, flamed cover-glass. A special slide having either a circular pit or a hollow-ground depression in the center is first lined with petrolatum on the surface around the edge of the depression, then inverted over the cover-glass in such a way that the droplet lies within the depression. The preparation is then turned over and examined under the microscope. The petrolatum serves the double purpose of preventing evaporation of the liquid and consequent development of convection currents within the droplet, and of an adhesive between cover-glass and slide. Bacteria grown on semisolid media may, of course, be suspended in a drop of physiological salt solution (0.85 per cent NaCl in distilled water) or nutrient broth on the cover-glass. Examinations for motility are best made on cultures not older than twenty-four hours and preferably on those which have been incubated twelve to eighteen hours. Unstained bacteria are somewhat difficult to see because of small differences between their refractive indices and that of the medium in which they are suspended. The diaphragm should be closed to a small aperture, and it is best to focus on the edge of the droplet first.

True motility must be differentiated from "brownian movement." When observed suspended in a liquid medium, bacteria and other particles of similar magnitude dance about rapidly, the agitation a result of continuous bombardment by the molecules of the suspending liquid. True motility, however, results in a different kind of motion. While non-motile bacteria are in a state of constant agitation, they remain within a circumscribed volume, drifting only slowly from one part of the microscopic field to another. The motile organisms, on the other hand, very definitely go from one place to another, across the field and in and out of focus. They

often move with surprising rapidity, crossing the field of vision in one or two seconds.

The processes of cellular division may also be observed microscopically by means of the so-called "hanging block." A small square of sterile nutrient agar is cut from a Petri dish containing the hardened medium and inoculated very lightly in the center. A cover-glass, sterilized by flaming, is placed on the inoculated surface and sealed by searing the edges of the agar with a hot needle, and the whole is sealed to a moist chamber with paraffin. The organisms are held in one position on the surface of the solid medium, and the processes of cell division may be followed readily.

Stained Bacteria.[17]—Bacteria in the unstained state are difficult to see. It was the development of staining techniques by Koch and others that made possible the ready observation of these organisms. The process is essentially one of dyeing the organisms with aniline dyes, the basic dyes being the most useful. Those commonly used are, in order of merit, gentian violet, methylene blue and basic fuchsin. Both gentian violet and fuchsin stain bacteria relatively rapidly, while methylene blue acts somewhat more slowly. The last is particularly useful, in the form of Löffler's alkaline methylene blue, for staining bacteria in pus, throat smears and similar materials. These stains are most conveniently kept in saturated alcoholic solution. The various staining solutions may be readily prepared from such stock solutions. The precise formulae for staining solutions of these and other dyes as well as those of special stains, some of which are described below, may be found elsewhere.[18]

There has been some controversy over the nature of the staining process, as to whether it is the result of a physical or chemical affinity between the dye and the bacterial cell. More recent data suggest that it is an adsorption exchange process primarily chemical in nature.[19]

The slides used for the preparation of smears should be clean and free from all grease. A preliminary soap and water cleaning may be necessary, and the cleaned slides are stored in alcohol or xylol. Before use a slide is wiped with a clean soft linen cloth and passed through the bunsen burner flame two or three times. A drop of distilled water is placed upon the slide and a small amount of bacterial growth suspended in it. The two are thoroughly mixed, and the suspension is spread over a larger area of the slide and allowed to dry in air. Films may be prepared from broth cultures, but these usually contain considerable quantities of extraneous material. After drying, the film may be fixed by passing through the bunsen burner flame two or three times. Films may also be fixed by immersion in absolute methyl alcohol, glacial acetic acid or other fixatives. The fixed film is covered with the staining solution, allowed to stand for fifteen to thirty seconds (a minute or more is desirable with methylene blue) and

[17] Further details may be found in *Biological Stains*. Prepared by the Commission on the Standardization of Biological Stains. H. J. Conn, Chairman. 3rd edition. Geneva, N. Y. 1940.

[18] Manual of Methods for the Pure Culture Study of Bacteria. Society of American Bacteriologists. Geneva, N. Y. 1923 *et seq.* Or any good laboratory manual.

[19] See the discussion by McCalla: Stain Technol., 1941, *16:*27.

washed in clean water. The stained film may be partially dried by blotting and the drying completed in air. It may be examined under the oil immersion lens directly, the cedar oil being placed on the stained film. If a permanent mount is desired, the cedar oil may be removed by flooding the slide with xylol, drying and applying balsam and a cover-glass.

The Gram Stain.—The procedure used in the preparation of a simple stain may be varied and elaborated for special purposes. In some cases bacteria exhibit differences in their reaction to stains, differences which may be taken advantage of in the differentiation of certain species. One of these elaborated procedures is known as Gram's method and the resulting preparation as a gram stain. A variety of modifications of Gram's original method have been introduced, most of which have their adherents. In general, the procedure consists of staining a fixed film with crystal violet solution containing ammonium oxalate, washing this off with water and flooding with Lugol's iodine solution. The iodine solution is washed off after a time and the film decolorized with alcohol, acetone, acetone and ether, or some other agent and counterstained with a dye of contrasting color, such as safranine 0 or Bismarck brown. By this means, bacteria may be divided into two groups: those which retain the original crystal violet in spite of the decolorization and appear dark purple on a background of the counterstain and are spoken of as *gram-positive* bacteria; and those which are decolorized and are colored by the counterstain and are called *gram-negative*. Certain bacteria are gram-variable, sometimes retaining the stain and sometimes not, but this group is not large enough to detract from the practical differential value of the stain.

Both the crystal violet and the iodine are highly specific while the alcohol and the counterstain are not. Almost all other dyes, even methyl green which differs by only one methyl group from crystal violet, are unsuitable, either being retained or removed by alcohol whether or not the iodine is applied. A few other reagents, notably $HgCl_2$, may be substituted for iodine, and stannous chloride, pyrogallol and freshly prepared solutions of hydroquinone show some slight activity.

A number of precautions must be observed in staining bacteria by Gram's method. The procedure is obviously an arbitrary one, and the instructions regarding the times the various solutions are allowed to act must be followed explicitly. Gram-positive organisms may lose the crystal violet if decolorized too vigorously, and *vice versa.* Young cultures, twenty-four to forty-eight hours old, should be used, for old cultures of gram-positive bacteria often contain many gram-negative cells.

The Mechanism of the Gram Stain.—However arbitrary Gram's method of staining may appear, the reaction is apparently associated with fundamental differences between the gram-positive and gram-negative organisms. There is, for example, a pronounced correlation between it and resistance to the bacteriostatic action of certain dyes (p. 68) and antibiotic substances (p. 132), and to the action of other chemical and physical agents. The nature of the gram reaction has, therefore, been of considerable interest and three general types of theories to explain the differential staining have been developed:

(1) The theory of differential membrane permeability was proposed by Benians,[20] whose experimental evidence was consistent with the view that while the cell membrane of both gram-positive and gram-negative bacteria is permeable to the crystal violet and iodine reagents, that of the former is not permeable to the alcohol-soluble dye-iodine complex and hence the color is retained.

(2) The colloidal chemical theory was advanced by Stearn and Stearn[21] who postulated a relationship between the isoelectric point of the cell protoplasm and its affinity for basic dye on the basis of the negative response of gram-positive bacteria at acid *p*H's, and related evidence; the isoelectric point of gram-positive bacteria is at a lower *p*H than that of the gram-negative bacteria and therefore the former have a stronger affinity for the basic dye.

(3) A morphological theory was developed principally by Churchman.[22] His evidence strongly supported the view that the protoplasm of all bacteria is gram-negative and that the gram-positive forms are covered by a sheath or envelope of gram-positive material, *i.e.*, the gram-positive cell consists of a gram-negative "medulla" and a gram-positive "cortex."

More recent work has substantiated this third hypothesis. Henry and Stacy[23] were able to remove an outer layer from gram-positive bacteria by treatment with bile to leave a gram-negative cell or "cytoskeleton." The bile extract, not in itself gram-positive, contained protein, polysaccharide and the magnesium salt of ribonucleic acid. This material could be deposited on the surface of the extracted cells to render them gram-positive again, but could not be deposited on normally gram-negative bacteria. The ribonucleate appeared to be highly specific in that related compounds or other salts of ribonucleic acid could not be substituted for it. This work has been confirmed and extended by Bartholomew and Umbreit[24] who converted gram-positive bacteria to gram-negative forms by digestion with ribonuclease and, further, were able to replace the bacterial ribonucleate with magnesium ribonucleate prepared from yeast. There was also evidence that the sulfhydryl groups of the protein were involved in some way in the gram reaction as a whole.

The gram reaction, therefore, appears to depend on a surface structure of magnesium ribonucleate combined with protein which is present on gram-positive bacteria but lacking on gram-negative bacteria. This explanation is consistent also with the evidence brought forward by others but interpreted differently; for example, the reaction between the crystal violet and iodine reagents and the ribonucleate-protein complex does not occur at acid reactions, hence the reversal of the gram-positive reaction by lowering *p*H.

[20] Benians: Jour. Path. Bact., 1912, *17*:199; *ibid.*, 1920, *23*:401.

[21] Stearn and Stearn: Jour. Bact., 1924, *9*:463, 479.

[22] Churchman: Jour. Exp. Med., 1927, *46*:1009; Jour. Bact., 1929, *18*:413; also in Jordan and Falk: *Newer Knowledge of Bacteriology and Immunology.* University of Chicago Press. Chicago. 1928.

[23] Henry and Stacy: Nature, 1943, *151*:671.

[24] Bartholomew and Umbreit: Jour. Bact., 1944, *48*:567. This paper includes a critical review of the pertinent literature.

Acid-fast and Spore Stains.—Both bacterial spores and a group of bacteria known as the acid-fast organisms are relatively difficult to stain. The staining reaction may be accelerated by heat, and such structures and organisms will take a dye—fuchsin is commonly used—either when it is applied hot for one or two minutes or cold for a prolonged period (twenty-four hours). Conversely, the dye is equally difficult to remove, and both spores and acid-fast bacteria, once stained, are not decolorized readily by such various agents as acid alcohol (1 per cent hydrochloric acid) or dilute (1 to 2 per cent) hydrochloric or sulfuric acid—hence the term acid-fast. The demonstration of acid-fast bacteria, such as the tubercle bacillus, consists, therefore, of staining with hot fuchsin containing carbolic acid followed by decolorization with dilute acid and counterstaining with a contrasting dye such as methylene blue. This technique is known as the Ziehl-Neelsen carbol-fuchsin stain. A variety of similar methods has been developed for staining bacterial spores, many of which include the use of a mordant such as chromic acid. In any case, the stained preparations show the bacilli or spores as cherry-red and the background, including other bacteria, in contrasting blue.

The capsule surrounding some bacteria may be stained directly by gentian violet or demonstrated by the so-called relief stain. In the relief stain the organisms are suspended in a drop of diluted (1 : 4) India ink and the mixture is spread across the slide with the edge of a second slide. The film should be uniformly spread and gray in color. After drying and fixation, the organisms may be stained with any of the usual stains. Upon examination the stained bacteria will be found to lie within lacunae in the film of ink, the capsule being delimited on the outside by the ink and on the inside by the stained bacterium.

The demonstration of tenuous organisms such as some of the spirochetes or the even more tenuous flagella, or organs of locomotion present on some bacteria, requires somewhat more elaborate procedures in which dye or silver is precipitated on the organism. The flagella, or organisms in the case of the spirochetes, appear after staining to be considerably larger than they actually are in the living state and are, therefore, readily seen. Many such methods have been proposed, the details of which may be found elsewhere.

The general question of staining should not be left without mention of the so-called polychrome stains. These stains consist of more than one dye in the same solution. Pappenheim's stain, for example, contains methyl green and pyronine and, when applied to pus smears, stains bacteria bright red and the nuclei of leucocytes blue or purple. Wright's stain, a combination of the basic dye methylene blue and eosin, an acid dye, is extremely useful for blood smears. Giemsa's stain, containing other dyes, is useful in the staining of pus and exudates and is extensively employed in the staining of protozoan parasites such as trypanosomes. In general, the polychrome stains are most useful for staining smears of pus, exudates of various kinds and other body fluids containing microorganisms.

Study of Bacteria in Pure Culture.—It will be apparent at this point that bacteria may be studied in a variety of ways. Only the com-

mon methods have been described here. Others, such as the immunological methods, are discussed elsewhere. Clearly, the characteristics of bacteria that are of significance in their identification must be studied in some uniform fashion, not only with regard to the methods used but also as to the characteristics that shall be considered of first importance. Although the classification of bacteria is in a somewhat dubious state, this uncertainty has resulted largely from a relatively hazy comprehension of the fundamental biological significance of some differential characteristics and consequent doubt as to the proper relative weight to be assigned to them in the arrangement of the finer subdivisions of taxonomic schemes. The main lines of approach are, however, clear, and whatever their relative biological significance may prove to be, the readily determinable characteristics of bacteria serve the practical purpose of distinguishing these microorganisms from one another.

There are four general categories which, in the order of the fineness of distinction, they make possible: (*a*) morphology, both gross and microscopic; (*b*) physiological capabilities in terms of biochemical reactions: (*c*) pathogenicity for experimental animals; (*d*) immunologic character. Of these the third is, for the most part, a matter of the pathology of diseases caused by bacteria and is necessarily discussed under the specific disease. Immunological differentiation of otherwise closely related groups of bacteria is likewise discussed elsewhere.

The preliminary and basic study of a bacterium lies in the systematic determination of its cultural characters. The most important of these are:

(*A*) morphology
- 1. gross morphology—that of colonies of the organism with respect to size, texture, color, shape, etc.
  - (*a*) on nutrient or infusion semisolid media
  - (*b*) on special media
- 2. microscopic morphology, including
  - (*a*) size, shape and grouping of the organisms
  - (*b*) presence or absence of spores
  - (*c*) motility
  - (*d*) presence or absence of capsule
  - (*e*) staining reactions

(*B*) biochemical reactions
- 1. the fermentation of sugars, usually dextrose, lactose and sucrose, although others may be included together with the hydrolysis of starch
- 2. liquefaction of gelatin
- 3. formation of indol
- 4. reduction of nitrate to nitrite
- 5. production of hydrogen sulfide
- 6. special biochemical tests such as the Voges-Proskauer reaction, the methyl red test, hemolysis on blood agar, etc.

Such preliminary examination ordinarily affords a great deal of information about a given bacterial culture. The feasibility of additional study rests firmly on the foundation laid down by the studies outlined above.

Various attempts have been made to systematize the study of bacteria. One of the most successful of these has resulted from the efforts of a Committee on Bacteriological Technique of the Society of American Bacteriol-

Name of organism .... Source .... Studied by .... Culture No. ....

Date of isolation .... Habitat .... Optimum conditions: Media .... Temp. ....°C.

Is phase variation observed? .... Phase on this Chart: S, R, M, G (smooth, rough, mucoid, gonidial) .... Phases recorded on other charts: ....

Underscore required terms.

SKETCHES

VEGETATIVE CELLS: Medium used........................
Reaction (pH)............Temp............Age............d.
Size of Majority........................
Ends, *rounded, truncate, concave, tapering*

MOTILITY: In broth............ On agar............

SPORANGIA and ENDOSPORES: *present, absent.*
Medium used............pH............Temp............Age............d.
Endospore Form: *spherical, ellipsoid, cylindrical*

IRREGULAR FORMS:
Present on............in............days at............°C.

AGAR COLONIES: Temperature............°C. Age........d.
Form, *punctiform (i. e. under 1 m.m. diam.), circular (i. e. over 1 m.m. diam.), filamentous, irregular, rhizoid.*
Surface, *smooth, rough, concentrically ringed, radiately ridged.*
Edge, *entire, undulate, lobate, erose, filamentous, curled.*
Elevation of growth, *effuse, flat, raised, convex.*
Optical Characters, *opaque, translucent, opalescent, iridescent.*

Surface Colonies | Deep Colonies

GELATIN COLONIES: Temperature............°C. Age........d.
Form, *punctiform, circular, irregular, filamentous.*
Elevation, *flat, raised, convex, pulvinate, crateriform (liquefying).*
Edge, *entire, undulate, lobate, erose, filamentous, curled.*
Liquefaction, *cup, saucer, spreading.*
Surface, *smooth, contoured, rugose.*
Optical Characters, *opaque, translucent, opalescent, iridescent.*

Surface Colonies | Deep Colonies

AGAR STROKE: Temperature............°C. Age........d.
Growth, *scanty, moderate, abundant, none.*
Form of growth, *filiform, echinulate, beaded, spreading, arborescent, rhizoid.*
Lustre, *glistening, dull.*
Chromogenesis........................*photogenic, fluorescent.*
Odor, *absent, decided, resembling*........................
Consistency, *butyrous, viscid, membranous, brittle.*
Medium, *grayed, browned, reddened, blued, greened, unchanged.*

Medium: Temperature ....°C. Age ....d.

NUTRIENT BROTH: Temperature............°C. Age........d.
Surface growth, *ring, pellicle, flocculent, membranous, none.*
Clouding, *slight, moderate, strong, transient, persistent, none, fluid turbid, granular growth.*
Odor, *absent, decided, resembling*........................
Sediment, *compact, flocculent, granular, flaky, viscid.*
Amount of sediment, *abundant, scanty, none.*

Medium: Temperature ....°C. Age ....d.

GELATIN STAB: Temperature............°C. Age........d.
Growth, *uniform, best at top, best at bottom.*
Line of puncture, *filiform, beaded, papillate, villous, arborescent.*
Liquefaction, *none, crateriform, infundibuliform, napiform, saccate, stratiform:* begins in............d. complete in............d.
Degree of liquefaction in............days........................
Method used........................
Medium, *fluorescent, browned, unchanged.*

Medium: Temperature ....°C. Age ....d.

FERMENTATION — Temperature............°C.

	Monosaccharides							Disaccharides						Polysaccharides						Alcohols							Glucosides				
Medium............ containing............ ....and:	Arabinose	Rhamnose	Xylose	Glucose	Fructose	Galactose	Mannose	Lactose	Sucrose	Maltose	Trehalose	Melibiose	Cellobiose	Raffinose	Melezitose	Starch	Inulin	Dextrin	Glycogen	Glycerol	Erythritol	Arabitol	Adonitol	Mannitol	Sorbitol	Dulcitol	Salicin	Aesculin	Coniferin	a-Methyl Gluc.	
Gas in............ fermentation tube																															
Amt. CO_2 in Eldredge tube																															
Reaction (pH) after............d.																															
Titrable acidity in ml. of N/......NaOH																															

BRIEF CHARACTERIZATION

As each of the following characteristics is determined, indicate in proper marginal square by means of figure, as designated below. In case any of these characteristics are doubtful or have not been determined, indicate with the letters U, V, and X according to the following code:
U, undetermined; V, variable; X, doubtful.

MORPHOLOGICAL

Vegetative Cells
- Form & arrangement: 1, streptococci; 2, diplococci; 3, micrococci; 4, sarcinae; 5, rods; 6, commas; 7, spirals; 8, branched rods; 9, filamentous
- Diameter: 1, under 0.5μ; 2, between 0.5μ and 1μ; 3, over 1μ
- Gram stain: 0, negative; 1, positive
- Flagella: 0, absent; 1, peritrichic; 2, polar; 3, present but undetermined
- Capsules: 0, absent; 1, present
- Chains (4 or more cells): 0, absent; 1, present

SPORANGIA: 0, absent; 1, elliptical; 2, short rods: 3, spindled; 4, clavate; 5, drumsticks

ENDOSPORES: 0, absent; 1, central to excentric; 2, subterminal; 3, terminal

CULTURAL

Agar Stroke
- Growth: 0, absent; 1, abundant; 2, moderate; 3, scanty
- Lustre: 1, glistening; 2, dull

Agar Colonies
- Form: 1, punctiform; 2, circular (over 1 mm. diameter); 3, rhizoid; 4, filamentous; 5, curled; 6, irregular
- Surface: 1, smooth; 2, contoured; 3. rugose

Gelatin Colonies
- Form: 1, punctiform; 2, circular (over 1 mm.); 3, irregular; 4, filamentous
- Surface: 1, smooth; 2, contoured; 3, rugose

PHYSIOLOGICAL

Biologic relationships: 1, pathogenic for man; 2, for animals but not for man; 3, for plants; 4, parasitic but not pathogenic; 5, saprophytic; 6, autotrophic

Relation to free oxygen: 1, strict aerobe; 2, facultative anaerobe; 3, strict anaerobe; 4, microaerophile

In nitrate media: 0, neither nitrite nor gas; 1, both nitrite and gas; 2, nitrite but no gas; 3, gas but no nitrite

Chromogenesis: 0, none; 1, pink; 2, violet; 3, blue; 4, green; 5, yellow; 6, orange; 7, red; 8, brown; 9, black

Other photic characters: 0, none; 1, photogenic; 2, fluorescent; 3, iridescent

Indole: 0, negative; 1, positive

Hydrogen sulfide: 0, negative; 1, positive

Hemolysis: 0, negative; 1, positive

Methemoglobin: 0, negative; 1, positive

Protein Liquefaction or Digestion
- Gelatin: 0, negative; 1, positive
- Casein: 0, negative; 1, positive
- Egg albumin: 0, negative; 1, positive
- Blood serum: 0, negative; 1, positive

Indicator Reduction
- Litmus: 0, negative; 1, positive
- Methylene blue: 0, negative; 1, positive
- Janus green: 0, negative; 1, positive

Rennet production: 0, negative; 1, positive

SUPPLEMENTARY DATA

TEMPERATURE RELATIONS

Medium........................pH........
Optimum temperature for growth....................°C.
Maximum temperature for growth....................°C.
Minimum temperature for growth....................°C.
THERMAL DEATH POINT: Time 10 minutes:..............°C.
Medium........................pH........
THERMAL DEATH TIME:
Medium........................pH........

Temp.	Time	Temp.	Time
.......°C.	.......min.	.......°C.	.......min.
.......°C.	.......min.	.......°C.	.......min.
.......°C.	.......min.	.......°C.	.......min.
.......°C.	.......min.	.......°C.	.......min.
.......°C.	.......min.	.......°C.	.......min.

CHROMOGENESIS

Gelatin..............................
Agar..............................
Potato..............................
....

OTHER PHOTIC CHARACTERS

Photogenesis on..............................
Iridescence on..............................
Fluorescence in..............................
....

RELATION TO REACTION (pH) OF MEDIUM

Medium..............................
Optimum for growth: *about pH*..............................
Limits for growth: *from pH*..............*to*..............

RELATION TO FREE OXYGEN

Method..............................
Medium..............................Temp..........°C.
Aerobic growth: *absent, present, better than anaerobic growth, micro-aerophilic*
Anaerobic growth: *absent, occurs in presence of glucose, of sucrose, of lactose, of nitrate; better than aerobic growth*
Additional data:..............................
....
....

MILK

Temperature..........°C.

Reaction:	.....d. ......;	.....d. ......;	.....d. ......
Acid curd:	.....d. ......;	.....d. ......;	.....d. ......
Rennet curd:	.....d. ......;	.....d. ......;	.....d. ......
Peptonization:	.....d. ......;	.....d. ......;	.....d. ......

LITMUS MILK

Temperature..........°C.

Reaction:	.....d. ......;	.....d. ......;	.....d. ......
Acid curd:	.....d. ......;	.....d. ......;	.....d. ......
Rennet curd:	.....d. ......;	.....d. ......;	.....d. ......
Peptonization:	.....d. ......;	.....d. ......;	.....d. ......

Reduction of litmus *begins in*.........*days, ends in*.........*days*

ACTION ON ERYTHROCYTES

Cells:..............................
Method: *plate, broth, filtrate*
Hemolysis: *negative, positive*
Methemoglobin: *negative, positive*

PRODUCTION OF INDOLE

Medium..............................
Test used..............................
Indole *absent, present in*..............................*days*

PRODUCTION OF HYDROGEN SULFIDE

Medium..............................
Test used..............................
H_2S *absent, present in*..............................*days*

ACTION ON NITRATES

Medium..............................Temp..............°C.

Nitrite:	....d. .....;	....d. .....;	....d. .....;	....d. .....
Gas (N_2):	....d. .....;	....d. .....;	....d. .....;	....d. .....

Medium..............................Temp..............°C.

Nitrite:	....d. .....;	....d. .....;	....d. .....;	....d. .....
Gas (N_2):	....d. .....;	....d. .....;	....d. .....;	....d. .....

Ammonia production (in amino-N-free nitrate medium): *negative, positive*
Complete disappearance of nitrate in..............medium: *negative, positive*
Disappearance of 2 p.p.m. nitrite in..............medium: *negative, positive*

REDUCTION OF INDICATORS

Medium..............................pH.........Temp..........°C.

Indicator	Conc.	Reduction:	
....	.......%	.....hr. .....;	.....hr. .....
....	.......%	.....hr. .....;	.....hr. .....
....	.......%	.....hr. .....;	.....hr. .....
....	.......%	.....hr. .....;	.....hr. .....

STAINING REACTIONS

Gram: .....d. .....; .....d. .....; .....d. .....; .....d. .....
Method..............................
Spores: Method..............................
Capsules: Method..............................
Medium..............................
Flagella: Method..............................
Special Stains:..............................
....
....

ADDITIONAL TESTS

Methyl red: *negative, positive*
Voges-Proskauer: *negative, positive*
Growth in sodium citrate: *absent, present*
Growth in uric acid: *absent, present*
Hydrolysis of starch: *complete (iodine colorless); partial (iodine reddish-brown); none (iodine blue)*
Nitrogen obtained from the following compounds:..............................
....
....
....

PATHOLOGY

ANIMAL INOCULATION

Medium used..............................Age of culture..............Amount..............Incubation period..............

		Whole culture			Cells			Filtrate		
Animal										
Type of Injection	Subcutaneous	*								
	Intraperitoneal									
	Intravenous									
	Per os									

*In each instance where pathogenicity is observed, indicate location of lesion, and type, e. g. edema, histolysis, gas, hemorrhage, ulcer, diphtheritic, etc.

ANTIGENIC ACTION

Animal..............................Medium used..............................Age of culture..............
Type injection..............................Number of injections..............
Culture causes production of *cytolysins, agglutinins, precipitins, antitoxin.*
Specificity: Antibodies produced effective against other antigens as follows..............................
....
Immune sera from..............................
..............................effective against this organism as antigen

SPECIAL TESTS

This DESCRIPTIVE CHART presented at the annual meeting of the SOCIETY OF AMERICAN BACTERIOLOGISTS, Dec. 28, 1934, by the Committee on Bacteriological Technic. Prepared by a sub-committee consisting of M. W. Jennison and H. J. Conn.

Name of organism..Studied by..Culture No..........................

Source..Habitat..Date..........................

Descriptions (*Underscore required terms.*)	Sketches
CELL MORPHOLOGY Medium: Vegetative cells: Age: Form and arrangement: *streptococci, diplococci, micrococci, sarcinae, rods, commas, spirals, branched rods, filaments.* Motility in broth: Flagella: Size: Irregular forms: Sporangia: *none, rods, spindles, elliptical, clavate, drumstick.* Age: Endospores: Shape: *spherical, ellipsoid, cylindrical.* Position: *central to excentric, terminal, subterminal.*	Temp. °C.
STAINING CHARACTERISTICS Gram: Age: Method: Special stains:	
AGAR STROKE Age: Amount of growth: *scanty, moderate, abundant.* Form: *filiform, echinulate, beaded, spreading, rhizoid.* Consistency: *butyrous, viscid, membranous, brittle.* Chromogenesis: ; *fluorescent, iridescent, photogenic.*	Temp. °C.
AGAR COLONIES Age: Form: *punctiform, circular, filamentous, rhizoid, irregular.* Elevation: *effuse, flat, raised, convex.* Surface: *smooth, contoured, radiate, concentric, rugose.* Margin: *entire, undulate, erose, filamentous, curled.* Density: *opaque, translucent.*	Temp. °C.
NUTRIENT BROTH Age: Surface growth: *none, ring, pellicle, flocculent, membranous.* Subsurface growth: *none, turbid, granular.* Amount of growth: *scanty, moderate, abundant.* Sediment: *none, granular, flocculent, viscid, flaky.*	Temp. °C.
GELATIN STAB Age: Liquefaction: *none, crateriform, infundibuliform, napiform, saccate, stratiform.* Rate: *slow, moderate, rapid.*	Temp.
OTHER MEDIA Age:	Temp.

FERMENTATION	Temp. °C.				
Medium: Carbohydrate: % Indicator:	Glucose	Lactose	Sucrose		
Acid in days					
Acid in days					
Gas in days					
Gas in days					

ACTION ON MILK	Temp. °C.					
Indicator:	Days					
Reaction						
Acid curd						
Rennet curd						
Peptonization						
Reduction (before coagulation)						

ACTION ON NITRATES

Medium: Temp. °C.

Nitrite: ....d. ; ....d. ; ....d.

Gas (N): ....d. ; ....d. ; ....d.

HYDROGEN SULFIDE PRODUCTION

Medium: Age:

H_2S: *present, absent.* Temp. °C.

TEMPERATURE RELATIONS

Growth in refrigerator (°C.): *present, absent.*
Growth at room temperature (°C.): *present, absent.*
Growth at 37° C.: *present, absent.*
Growth at 50° C.: *present, absent.*

INDOLE PRODUCTION

Medium: Age:

Method: Temp.

Indole: *present, absent.*

RELATION TO FREE OXYGEN

Medium: Age:

Method: Temp. °C.

Aerobic growth: *absent, present, better than anaerobic growth, poorer than anaerobic growth.*

Anaerobic growth: *present, absent.*

ADDITIONAL TESTS

ogists, under whose auspices a Manual of Pure Culture Study has been published and is supplemented by leaflets from time to time. Through this agency standard methods and descriptive terminology are made available. Furthermore, the same committee has prepared a standard descriptive chart for the recording of data, and from time to time the chart is revised.

Those organisms which separate completely after cell division and appear singly and scattered at random over the microscopic field are designated as *micrococci.* In some species, however, there is a tendency for the individual cells to remain in pairs, and one may observe such pairs intermingled with individual cells. These organisms are called *diplococci.* When this pairing tendency is marked as, for example, in the gonococcus, the individual cells may not be perfectly spherical but exhibit a coffee bean shape with the concave sides facing each other. Often coccus forms tend to remain together in sheets or irregular clusters resembling bunches of grapes. Those which assume this grouping are termed *staphylococci.* Division in two or three planes is, of course, necessary to the formation of such groups. Other coccus forms divide in only one plane but tend to remain together, the result being a chain of cocci. Organisms forming such chains are called *streptococci.* Still other cocci divide in three planes and remain together to form cubical packets of eight cells. These organisms are the

Fig. 4.—Morphologic types of bacteria. *A*, micrococcus; *B*, diplococcus; *C*, staphylococcus; *D*, streptococcus; *E*, sarcina; *F*, coccobacilli: *G* and *H*, commonly occurring rod forms; *I*, fusiform bacilli; *J*, curved rods or vibrios; *K* and *L*, spiral forms.

sarcinae. Not all the cells in a given microscopic field will appear in characteristic groups. Many organisms may be found singly, presumably those which have broken away from the parent group. Chain formation by streptococci is influenced by the medium upon which the organisms have been grown. The acid-producing streptococci of milk, for example, show long chains in milk culture but when grown in nutrient broth may show only a few short chains. In smears of pus or similar material, however, the coccus forms are usually found in their characteristic groupings. Of the coccus forms having definite grouping, the staphylococci are the most consistently characteristic under the microscope.

Bacillus.—The morphology of individual rod-shaped bacteria differs considerably from species to species. Not only is there variation in size but the shape of the individual cells differs. Some bacilli have sides more or less parallel with one another but rounded ends. Others have quite square ends which appear to have been cut off sharply. Still others as-

sume a highly elongated but oval shape. These last, when short and thick, are often called *coccobacilli.* A given shape is relatively constant within a species although the length-width ratio may vary considerably. The groupings which the cells may assume are a result of post-fission movements, for the plane of division varies rarely if at all. The tendency to remain attached, end to end, resulting in the formation of a chain of *streptobacilli,* is more or less constant for some species but is observed only occasionally in many species. Other groupings of these cells result from the movements of the bacilli after division is complete. Some tend to slide together side by side, a movement known as *slipping,* which results in the formation of palisade-like groups of cells. Still others, in a movement known as *snapping,* bend sharply at the point of division to an acute angle and present a V-shaped appearance. When this type of movement is associated with a tendency for the bacilli to remain attached

Fig. 5.—Electron micrographs of bacteria. Left, the tubercle bacillus, × 26000; note the granular structure of the cell. Right, *Micrococcus flavus,* × 16000; note the homogeneity of these cells and the lack of evidence of internal structure (von Borries).

end to end, chains of organisms resembling a split rail fence may result. Such snapping often results in breakage of a chain of organisms, the parts of which continue to grow in the new direction. The groupings assumed by bacilli are, however, not as characteristic as those of the cocci.

The Spiral Forms.—The third principal type of bacterial form commonly observed is that of the spiral or screw-shaped forms. There are a variety of these which may be distinguished morphologically, but at the moment only three main subtypes may be considered. The first of these is the *vibrio* or curved rod. Smears of such organisms often show chains which, since the organisms curve in alternate directions, suggest a spiral organism. It is not difficult, however, to distinguish between such chains and the truly spiral organisms. A spiral organism which is rigid, or relatively so, is called a *spirillum,* while similar organisms which are flexible are termed *spirocheles.* Further distinctions, such as the presence or ab-

sence of an undulating membrane, etc., which are made use of in formal taxonomic schemes, need not concern us here (see p. 162).

Involution Forms.—Although the vast majority of bacteria exhibit a marked constancy of form during the early stages of the growth of a culture, in older cultures aberrant forms such as over-sized cocci, Y-shaped bacillary forms and forms apparently containing considerable quantities of granular material may be found. These are generally regarded as *involution* or *degenerative* forms which are dead or dying and represent varying stages in the dissolution of the cell structure. Some workers, however, attach significance to such forms and consider them as indicative of the existence of complex life cycles. Such irregular bizarre forms may often be produced at will by cultivation of bacteria under somewhat adverse conditions of temperature, salt concentration and the like. Morphological variations and their implications are discussed at greater length elsewhere (Chap. 6).

The Structure of Bacterial Cells.[5]—The structure of bacterial cells, particularly their finer structure, has been of considerable interest since these organisms were first studied. They are so small, however, that the limitations imposed by the optical system employed become of considerable importance.

The Compound Microscope.—With the ordinary microscope objects are viewed by transmitted light and therefore must be sufficiently large to cast a shadow. This size is approximately half the wave length of the light used. The highest numerical aperture that is practical in the objective is 1.4. Such a lens, when used with monochromatic light with a wave length of 5460 Å or 546 mμ, will resolve a particle having a diameter of 0.2 μ. Objects somewhat smaller than this may be seen in that they are visible but are not resolved and neither size nor shape can be determined. Since many bacteria are no wider than 0.2 to 0.5 μ the hope of observing intracellular structures is a faint one. Some workers, Barnard[6] in particular, have attempted to extend the limits of resolution through the use of ultraviolet light and quartz lenses coupled with "seeing" by means of a photographic plate, but such efforts have not added greatly to knowledge.

The Darkfield or Ultramicroscope.—In the instrument known as the darkfield (*dunkenfeld*) or ultramicroscope, reflected rather than transmitted light is used, objects appearing brilliantly lighted against a black background. The usual microscope may be used as a darkfield by attaching a darkfield condenser in place of the Abbé substage condenser. The principle is the Tyndall effect, perhaps most commonly observed in the appearance of particles of dust in a shaft of sunlight in a darkened room. The limit of resolution is not increased, however, and while exceedingly small objects are visible, they appear only as brilliant points of light. The use of this type of microscope has not contributed materially to knowledge of the structure of the bacterial cell except with regard to the mode of action of flagella. It is, however, very generally used for the detection

[5] The cytology of bacterial cells is discussed at length by Lewis: Bact. Rev., 1941, 5:181; and by Knaysi: *Elements of Bacterial Cytology.* Comstock. Ithaca. 1944.

[6] Barnard: Lancet, 1925, ii:117.

and observation of very slender microorganisms, especially spirochetes as in exudate from a syphilitic chancre or leptospira in the blood from cases of leptospirosis.

The Electron Microscope.—The recently developed instrument known as the electron microscope operates on a different principle from the optical microscopes in that a stream of electrons is used rather than a beam of light. The electron stream is focused by means of magnetic "lenses," and the interposed object intercepts the stream to cast a "shadow" which is recorded on a photographic plate to give an electron micrograph.[7] Remarkable resolution is possible because of the minute size of the electron and sharp photographs may be obtained at magnifications of 30,000 diameters or more. Its application is somewhat limited in that the object must be prepared as a dry film on collodion and observed in a high vacuum; in consequence, living organisms cannot be studied. Application of the electron microscope to biological problems is still in its infancy and as yet has given only very limited new information. For example, nuclei, chromosomes, genes, etc. have not yet been demonstrated in the bacterial cell. See Fig. 5 and those in Chapter 38.

Certain structures of bacterial cells are, however, of sufficient magnitude to be seen and studied with the optical microscope. The most obvious of these are structures external to the cell—capsules and flagella.

Capsules.—Many bacteria, perhaps all, are provided with a capsule, which apparently originates from the outer layer of the cell membrane; in stained preparations it can sometimes be seen surrounding the cell like a halo. The capsules of bacteria are often one or two times as thick as the diameter of the cell itself and may appear to extend continuously over a chain of organisms or completely surround paired organisms such as diplococci. Cultures of capsulated bacteria are usually slimy and the growth is sticky and viscous. The capsular material is generally polysaccharide in nature, though some bacterial slimes may contain mucin-like proteins. Some of these forms may be a source of considerable annoyance in the sugar refining industries. The presence of a capsule is also associated with the virulence of a variety of pathogenic bacteria. Pneumococci, for example, which have no capsule are relatively avirulent but when capsulated are highly virulent. Anthrax bacilli are almost always found to be capsulated when observed in preparations made from animal tissues. The capsule appears to function as a bacterial defense against the activity of phagocytic cells of the body. The sheaths of some of the filamentous bacteria may possibly be a capsule in somewhat different form. The colorless sulfur bacteria which derive their energy through the oxidation of hydrogen sulfide to elementary sulfur deposit sulfur granules in their sheaths. Likewise the iron bacteria which oxidize iron from the ferrous to ferric state, deposit ferric hydroxide in the sheaths surrounding them.

Flagella.—Many kinds of bacteria are observed to be motile under the conditions in which bacteria are usually studied. Some of those forms

[7] The principles and construction of the electron microscope are reviewed by Zworykin: Science, 1940, *92*:51.

in which motion has never been observed may possibly possess the power of locomotion under certain unusual conditions. Independent bacterial motion is a true movement of translation, and is to be distinguished from the oscillating or quivering movement (brownian movement) exhibited by all very minute particles suspended in water or other fluids. Many bacteria are found to be motile when they are examined after removal from certain culture media, but non-motile if they have been grown on other substances. The colon bacillus, for example, is motile when picked from young colonies on gelatin or agar but is frequently non-motile when taken from broth. The rate at which a bacterium moves has been approximately measured. The typhoid bacillus may travel a distance of 4 mm., or about 2000 times its own length, in one hour; the cholera vibrio may attain a speed of 18 cm. per hour for short distances.

This power of locomotion depends upon the possession of flagella, long, fragile, filamentous, coiled appendages. The origin of flagella has been a matter of some interest.[8] It is generally assumed that they are extensions of the cell wall but Knaysi[9] believes, on the basis of electron micrograph

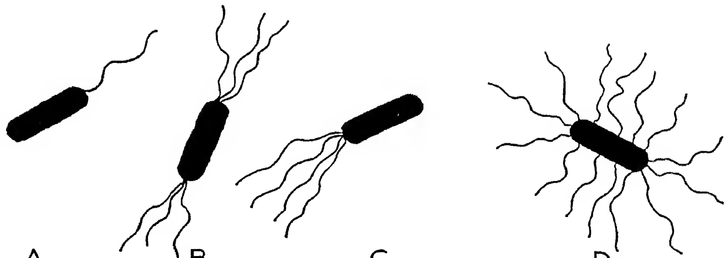


Fig. 6.—Position of flagella on the bacterial cell. *A*, monotrichous; *B*, amphitrichous; *C*, lophotrichous; *D*, peritrichous.

evidence, that their origin is the cytoplasm of the cell proper. These, by virtue of their power of contractility, drive the bacterium through the suspending medium. Flagella have been seen in the living, unstained cell (large spirilla) by some observers, but ordinarily special staining methods must be applied to reveal their presence. The propelling action is due, not to a lashing as once thought, but rather to a rhythmic contraction which moves helicoidally over the surface, the action being that of a screw rather than an oar.[10] According to Pijper[11] direction of movement is controlled by changes in the angle which the flagella make with the cell body, acting as a rudder.

Bacteria differ from one another with respect to the position of the flagella on the cell body. The *monotrichous* organisms have only a single flagellum at one end; the *amphitrichous* bacteria have either a single flag-

[8] *Cf.* the review by Knaysi: Bot. Rev., 1938, *4*:86, 99.

[9] Knaysi: Science, 1942, *95*:406.

[10] Reichert: Centralbl. f. Bakt., Abt. I, Orig., 1909, *51*:14; Neumann: *ibid.*, Abt. I, Orig., 1928, *109*:143.

[11] Pijper: Jour. Path. Bact., 1938, *47*:1.

ellum or a tuft of flagella at each end; the *lophotrichous* bacteria have a tuft of flagella at one end; and the *peritrichous* have flagella projecting from the whole body of the cell, the sides as well as the ends. Recent evidence has suggested that the occurrence of peritrichous flagellation is open to question. Pijper[11] has reported that such typically peritrichous forms such as the typhoid bacillus and *Proteus vulgaris* move by means of a long "tail" consisting of two flagella twisted together which are attached to the cell near the middle. According to Pietschmann[12] peritrichous flagellation is an illusion due to the appearance of subpolar flagella on chains of cells. Bacteria having no flagella are termed *atrichous*.

The number of flagella on the peritrichous bacteria varies considerably and even closely allied species may differ from one another in this respect. The typhoid bacillus, for example, has, as a rule, more flagella (ten to

Fig. 7.—Flagella of bacteria. Single polar flagella of a monotrichous bacterium (*Vibrio cholerae*) on the left, and peritrichous flagella (*Salmonella typhi-murium*) on the right; × 2000 (Kral).

twelve) than the colon bacillus (two to six). The majority of actively motile bacteria belong either to the bacilli or spirilla; very few cocci are motile under ordinary conditions. Within a given bacterial species different degrees of motility may be found. Some strains within a species may consist practically entirely of motile cells, while other strains may show no motility in hanging drop preparations nor may flagella be found in stained smears. Further, the motility of some strains is affected by the temperature of incubation while that of others is not; some strains may be motile when grown at 22° C. but not when grown at 37° C. Motility, therefore, is not a character that can be used for exact delimitation of species or varieties of bacteria. Standard cultural characters and biochemical reactions do not appear to be correlated with the presence or absence of motility.

Spores.—Some of the bacilli possess the ability to form resistant structures known as spores. Such spores, spherical or oval in shape, show a

[12] Pietschmann: Arch. f. Mikrobiol., 1939, *10:*133.

relatively high resistance to all sorts of injurious influences, including high temperatures, germicidal chemicals, etc. Furthermore, they stain with great difficulty. Usually heat in combination with a mordant is necessary to drive the dye into them.

An assembling or concentration of nuclear material (*i.e.*, material staining deeply with basic dyes) precedes spore formation in some cases and constitutes the spore primordium. The highly refractive character of the unstained spore and its reduced water content probably are connected with the concentrated character of the spore substance. Such spores may be formed in any part of the cell, the position generally being relatively constant within a species. The spore is spoken of as *terminal* if formed at one end of the cell, *central* if formed in the center of the cell, and *subterminal*

Fig. 8.—Spore formation by bacteria. Left, the anthrax bacillus which forms central spores which do not distend the wall of the vegetative cell; the tendency of the bacilli to occur in chains is also characteristic. Center, *Clostridium botulinum* type B, which shows the typical clostridial form with terminal spore of greater diameter than the vegetative cell; note the free spores separated from disintegrated vegetative cells. Right, *Clostridium sporogenes*, showing a subterminal clostridial spore. These preparations are all stained with a single stain in the usual way; the vegetative cells take up the dye but the spores remain unstained. Fuchsin; × 1050.

if formed half way between the center and the end of the vegetative cell In some cases the spore does not exceed the diameter of the parent cell, as in the anthrax bacillus; in others where its diameter is greater than that of the vegetative cell it causes a bulging out of the wall of the cell at the point where it lies. If such spores are terminal, a drum-stick appearance may result, as in the tetanus bacillus; or if it is central, the vegetative cell becomes spindle-shaped. In simple stains the spore appears as an unstained round or oval body lying within the stained protoplasm of the vegetative cell. When the spore is fully developed, the vegetative cell disintegrates and disappears.

The conditions under which bacteria form spores vary with the nature of the organism. The anthrax bacillus, an aerobe, forms spores only when in contact with free oxygen, a fact that has practical bearing on the dis-

posal of bodies of cattle dead of the disease. The tetanus bacillus, on the other hand, and the anaerobes in general, form spores in the entire absence of oxygen. A suitable temperature is essential to the formation of spores. The anthrax bacillus forms spores most abundantly at 30° C. to 32° C. and will not produce them at a temperature of 12° C. Lack of food is apparently not an adequate stimulus to spore formation. In all cases a period of uninterrupted vegetative multiplication precedes the appearance of spores, and the conditions necessary for their production seem to arrive simultaneously for most of the cells in a culture. In some cases the cause of spore formation is thought to be an accumulation of metabolic products in the culture. These may be acids or other injurious compounds. According to Daranyi[13] diminution in the water content of the bacterial cell, leading to a shrinking of the colloids, is the main factor in bringing about spore formation. Friedman and Henry[14] have found that the percentage of bound water is relatively high in spores as compared with vegetative cells, and suggest that this is associated with their heat resistance. In any case, spores are not formed under adverse conditions, as it might be thought, but in circumstances favorable to growth.

When spores are brought under conditions favorable for growth they *germinate* and become vegetative cells which multiply in the usual manner. As it undergoes germination the spore shows a change in its refractive properties, probably due to the imbibation of water. The entire spore wall may become thin and stretch and the spore assume a bacillary form, or a vegetative cell may burst through the spore membrane, the empty case being cast off as a hull. The anthrax bacillus grows out of one side of the spore wall; the closely related hay bacillus grows out of opposite sides simultaneously. Other forms of bacteria exhibit intermediate methods of germination. Also, irregularities may occur in the development of spores of the same species.

Spore formation is not very common among bacteria. It has been definitely observed only in the rod forms; cocci are not known to sporulate, and sporulating spirilla, if they exist, are rare. The majority of the obligate anaerobes produce spores, as do some of the aerobic bacilli. The spore-forming bacteria of known pathogenicity for man are few, a fortunate circumstance that materially facilitates and simplifies disinfection and the treatment of infectious disease. Physiologically, the spore is usually considered as a resting stage, serving to tide the species over unfavorable periods. From this point of view, the spore stage is analogous to the periods of hibernation or estivation among higher forms of life and the living matter of the spore may remain dormant for years or even decades. Spore formation is not a form of multiplication, for each vegetative cell forms only a single spore and each spore germinates into a single vegetative cell. Obviously no increase in numbers is possible by this process.

The Finer Structure of the Bacterial Cell.—Knowledge of the intracellular structures of bacteria is strictly limited, as pointed out above, by limits in the resolving powers of the microscope. Three questions have

[13] Daranyi: Centralbl. f. Bakt., 1927, Abt. II, *71*:353.

[14] Friedman and Henry: Jour. Bact., 1938, *36*:99.

been of interest, namely: the existence and nature of the cell membrane; the nature of the cell substance or entoplasm; and, finally, the question of the existence of a nucleus. In spite of many careful investigations by able workers, none of these has been satisfactorily elucidated, though partial answers are possible for all three.

The cell membrane of bacteria is not demonstrable as a structurally differentiated envelope, as that of many plant cells is, by immersion of the cells in hypertonic solutions. The interface or boundary between a bacterial cell and its environment is, of course, sharply defined, but there is no such differentiation between the entoplasm and the outer wall. The cell membrane is perhaps best regarded as a somewhat thickened and differentiated outer portion of the cell substance and possibly deserves the name ectoplasm rather than cell membrane. It would appear, however, to have considerable rigidity, particularly among the bacilli, in order to

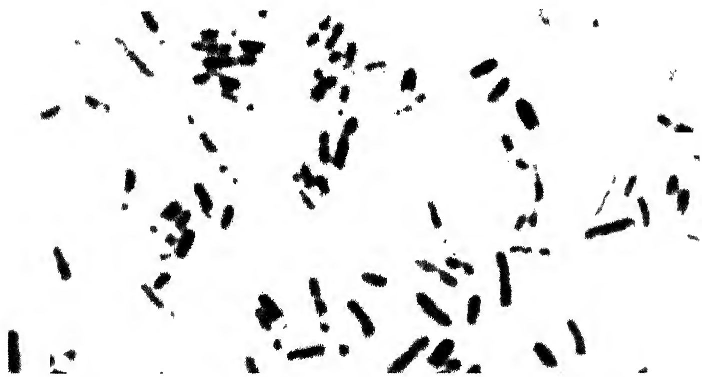


Fig. 9.—Irregular and bipolar staining of the plague bacillus. Note the heavily stained areas and metachromatic granules. Fixed in methyl alcohol and stained with methylene blue; × 2400.

maintain shapes other than spherical. Experiments on the growth of the colon bacillus in media in which the surface tension had been reduced by the addition of sodium oleate resulted in the production of long, filamentous forms, much longer than the usual rods. Such experiments have been interpreted as indicating that this organism is essentially tubular, the sides having sufficient rigidity to withstand the reduced surface tension but the length increasing because of a less rigid structure at the ends. Electron micrograph studies by Mudd and his associates[15] have indicated that the bacterial cell wall is a definite morphological structure from which the cytoplasm may shrink away, and which is sufficiently solid to show jagged lines of fracture when broken by sonic vibration. The precise nature of the bacterial cell membrane is of particular interest in connection with the problems of permeability.

[15] Mudd *et al.:* Jour. Bact. 1941, *41*:415; *ibid.*, 1941, *42*:251.

The nature of the cell substance is not well known. It is, of course, protoplasm, as the ancillary evidence of chemical composition indicates. The cells of actively growing cultures appear to be homogeneous both in the unstained state and when stained by various dyes. As the culture becomes older, however, the cells of many organisms may be found to contain granular material which is somewhat more refractive than the surrounding protoplasm in the unstained state, and which takes up certain dyes with greater avidity than the remainder of the cell. These granules are called *metachromatic granules* or, after the workers who first described them, Babes-Ernst granules. They are sometimes scattered through the cell substance, and sometimes massed at either end, where they constitute the "polar granules" observed in the plague bacillus, the glanders bacillus and certain other bacteria. In certain species the metachromatic granules are particularly easy to demonstrate, and their abundance may even

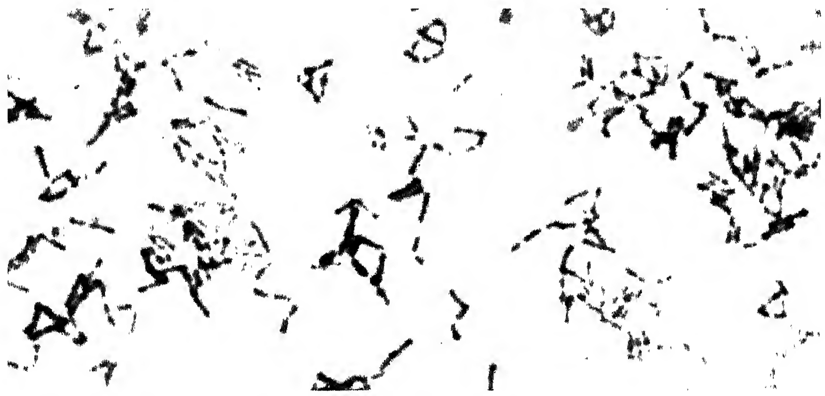


Fig. 10.—Metachromatic granules and bipolar staining in the diphtheria bacillus. Note the differences between these organisms and the plague bacilli. Methylene blue; × 1975.

constitute a character of some differential value. The nature of these granules has been the subject of microchemical investigations, but the results are somewhat vague. Some are lipoid in nature and others consist of polysaccharide material which gives a color with iodine reagents and has been designated *granulose*. The greater proportion of these granules, however, appear to consist of nucleoprotein and take up the nuclear stains with great avidity. This nucleoprotein has been termed *volutin*, and granules of this and other material are generally spoken of as "reserve material" —a term which, because of its implications, could hardly have been less wisely chosen.

The apparent lack of a discrete nucleus in the cells of the true bacteria has been a source of much speculation. Granular materials such as volutin and metachromatic granules which are characterized by staining deeply with basic dyes are sometimes referred to as "nucleoids" but apparently have no nuclear function. The seeming homogeneity of the cell substance,

both in the stained and unstained states, has been interpreted by some to indicate that these cells consist entirely of cytoplasm and have no nucleus, and by others to mean that these cells are all nucleus and have no cytoplasm. Little can be said in defense of either of these interpretations, and the fact that no other cells are known to exist without either cytoplasm or nucleus renders them both untenable. A third possibility remains, namely, that a discrete nucleus is contained within these cells but it has not been demonstrated by any technique as yet employed. Begging the question in this manner is, of course, entirely unsatisfactory. A fourth possibility, and one that is generally conceded to be the most acceptable, is that the bacterial cell contains nuclear material but that this material is scattered throughout the cell instead of taking the form of a discrete nucleus. Two lines of evidence support this theory: one, the detection by analytical means of not inconsiderable quantities of nucleic acids and nucleoprotein, substances known to be intimately associated with the discrete nuclei of other cells; and, second, the uniform staining of the bacterial cell with dyes known to have a marked affinity for nuclei, and the diffuse staining of the protoplasm by the Feulgen reagent, a reagent that gives a pink color with nuclear material and is said to be specific for the potential aldehyde groups of chromatin.[16] It may be said, therefore, that although they do not have morphologically discrete nuclei, *bacterial cells contain the chemical equivalent of a nucleus.*

Colonial Morphology.—A single bacterial cell or group of cells, when planted on a semisolid medium and allowed to develop under suitable conditions of moisture, temperature and air supply, will, in a few hours or days, develop a "colony" so large that it can be plainly seen with the naked eye. In many instances such masses of cells, particularly when the growth occurs on certain culture media, possess salient peculiarities which are characteristic of the species. Growths upon nutrient gelatin are especially characteristic, but upon nutrient agar the morphology of bacterial colonies is less distinctive. Colonies on potato and other solid food substances are, as a rule, still less characteristic. As was pointed out previously, colonial differences may often be accentuated by growth on media containing dyes and other compounds. Diphtheria bacilli, for example, form highly characteristic colonies on media containing potassium tellurite. The character of colonies is also somewhat affected by the density and viscosity of the culture medium and by the physical conditions under which the organisms develop. In general, however, the colony characteristics of a bacterial species are distinctive and are an aid in the isolation and identification of the organism. These characteristics include the size of the colony, its form—whether raised, flat, etc.—and the shape of its edges; its consistency and texture; its surface—whether roughened or smooth; its color; and other similar peculiarities.

These colony characteristics in many cases have their roots in the morphology of the individual cells. The movements of cells after fission and the arrangements that result determine, to a considerable extent, the texture of a colony and the shape of its edges. The long coiled filaments

[16] *Cf.* Barber and Price: Nature, 1940, *146*:335.

of the anthrax bacillus give the colony of this organism its characteristic "medusa-like" appearance. The presence of capsules on an organism gives its colonies a slimy consistency, and their absence a dry, roughened appearance. The colonies of some bacteria show coloring due to the elaboration of pigments by the organisms, but such pigments are not apparent in the individual cell under the microscope. Yellow and golden pigments are not uncommon, and others, including red, blue-green and violet are met with from time to time.

Much stress should not be laid in all cases upon the morphology of masses of bacteria as an aid in distinguishing different species. The mass morphology, like the individual morphology, is subject to wide variation under varying conditions of life, and can be regarded as only one item in the sum-total of characters that go to make up the concept of a bacterial species. One difference between colony types which has recently been discovered appears to be of deep-seated significance. In a number of bacterial groups it has been found that rough (R) and smooth (S) colonies will develop from a single, apparently pure, culture (p. 152). Under ordinary conditions, transfers from S and R colonies often breed true. The rough and smooth strains differ in virulence and other characteristics, and the marked and persistent association of colony characters with physiological and biochemical qualities may be of considerable biological significance.

Growth and Cell Division.—Although bacterial growth may be, and often is, assumed to be a simple process, both experimental evidence and theoretical considerations indicate that the problems of growth are relatively complex. It is convenient to consider bacterial growth under two general heads: first, the process of cell division or multiplication; and, second, the growth of bacterial populations.

By all odds the most common method of division is that of simple fission which divides the cell into approximately equal halves. Among bacilli and spirilla, cell division usually takes place at right angles to the long axis of the cell. The cocci may divide in one, two or three planes, thus giving rise to characteristic groupings such as chains, flat sheets or irregular masses, or cubical packets. Bacilli and spirilla show some elongation before division; cocci, as a rule, do not, although some exhibit an increase in the diameter of the cell without any alteration of its spherical form. The size which a single cell must reach before fission is, as among the higher forms of life, singularly constant for each species although, as will appear, some differences in this maximum size appear to be associated with the age of the culture.

While bacterial multiplication is commonly observed to take place by simple transverse fission, other modes of cell division may occur. Some forms, such as the diphtheria bacillus and the tubercle bacillus, show Y-like splitting and true branching characteristic of some of the higher fungi. Budding, similar to the budding of yeast cells, has been reported by some observers. More complex processes, such as the formation within a mother cell of a number of viable granules, called gonidia, which, upon liberation, develop into typical bacillary forms, have been reported by other workers.

Whether such observations belong in the realm of fact or fancy is open to question. It has been pointed out[17] that the structures reported by some workers are so small that they could not have been resolved by the microscope. It may be said that, although binary fission is by far the most common method of cell division among the bacteria, some species do exhibit branching and possibly budding may occur. The existence of other means of multiplication must be regarded for the present as not established.

Under favorable conditions cell division may take place quite rapidly, as short a time as twenty minutes elapsing between one division and another of the colon bacillus. This rapidity of cell division is sometimes referred to as if it were a peculiar quality of bacteria, but as a matter of fact the embryonic cells of many higher forms of life divide quite as rapidly as bacteria. The remarkable thing about bacterial cell division is not so much the rapidity with which one cell division succeeds another as the fact that a very short time suffices for the growth of the young cell to maturity. A young bacterial cell attains full size and acquires the capacity to produce, in its turn, an independent organism much sooner than most other forms of life. This rapid reproduction of distinct individuals is plainly different from the multiplication of embryonic cells among higher organisms.

The Growth of Bacterial Populations.– For obvious practical reasons bacteria are ordinarily manipulated and studied in groups consisting of large numbers of individuals. Likewise, bacterial growth is of practical significance, not in terms of one, two or three cell divisions, but as growth in populations which often reach a density of three to four hundred million cells per cubic centimeter of culture fluid. Growth of these organisms in culture may be considered from a variety of points of view, the particular one adopted being dependent upon the nature of a given investigation. A simple view that is commonly, if tacitly, taken assumes that growth may be described by the reaction

$$\text{culture medium} + O_2 \longrightarrow \text{bacterial protoplasm} + \text{oxidized substances},$$

which proceeds from left to right and eventually comes to an equilibrium. The reaction velocity constants may be determined either by the rate of disappearance of the reacting substances or the rate of appearance of end products. Although complicated by a variety of side reactions, the assumption of such a relation has been extremely useful in the studies on bacterial metabolism. The growth of autotrophic bacteria, for example, is conveniently measured in terms of the conversion of carbonate to the organic carbon of protoplasm. A somewhat more mechanistic concept is that of the instability of an inoculated culture medium which, after undergoing a series of complex changes, reaches a stable equilibrium at a considerably lower energy level. Such a concept is, of course, the basis of studies of the energy metabolism of bacteria. These and similar views are oversimplified and, although of undoubted value, present only limited aspects of the phenomena of bacterial growth.

[17] Kluyver and van Niel: Centralbl. f. Bakt., 1936, Abt. II, *94*:369.

The existence of bacteria in large aggregations or *populations* is of no little significance because *ipso facto* they are subject to the principles of population mechanics. The potential multiplication of these organisms by geometrical progression may be realized only up to a certain point. As numbers increase in the microcosm of the test tube, competition between individual organisms for foodstuffs, oxygen and the like progressively reduces the opportunity for further growth until a saturating population is reached.

If no increasingly effective retardation were operative, the potential increase in a population would be expressed by the relation

$$\frac{dY}{dt} = bY$$

where Y is equal to the number of individuals per unit volume and *b* to the potential rate of multiplication of each organism. When, however, there is a maximal possible population, K, this potential geometric increase is only partially realized, the extent of the realization depending on how near the size of the population is to its maximum at any g ven moment. Or, mathematically,[18]

$$\frac{dY}{dt} = bY\,\frac{K - Y}{K}$$

This equation is the differential equation of the logistic curve[19] the mathematical form of which is:

$$Y = \frac{K}{1 + e^{a + bx}}$$

This function, plotting as a symmetrical sigmoid curve, has been found to describe, with a high degree of precision, the growth of populations of a variety of organisms including man, yeasts, *Drosophila*, certain protozoa, etc.

If the numbers of bacteria in a growing culture are determined from time to time and plotted against time, the points fall on a similar sigmoid curve. The resulting curve is, however, asymmetrical in that the point of inflection is not half way between the upper and lower asymptotes but is considerably nearer the lower asymptote. This discrepancy between bacterial populations and populations of other organisms has not as yet been satisfactorily explained.

Partly as a consequence of this discrepancy and partly as a result of the large error inherent in the enumeration of bacteria, the treatment of bacterial growth curves has taken a slightly different path. Such curves are ordinarily not plotted arithmetically but on semilog paper; *i.e.*, the logarithms of the numbers of bacteria are plotted against time. This procedure has the advantage that not only are the errors of enumeration minimized but, when the organisms are multiplying at a geometric rate, the points fall on a straight line and the generation time determines the slope of this line. Furthermore, the numbers of viable bacteria, as deter-

[18] For a more detailed discussion of such rationalization the student is referred to Lotka: *Principles of Physical Biology*. Williams & Wilkins Company. Baltimore. 1925.

[19] Pearl and Reed: Proc. Nat. Acad. Sci., 1920, *6*:275.

mined by plate count, decline not long after the maximum population has been reached and the organisms appear to die off at a geometric rate. The equilibrium predicted by the logistic function is not realized unless experimental conditions are such that the food supply is constantly renewed and the waste products are removed. Such an equilibrium is attained, for example, in cultures in constantly flowing culture medium. A graphic representation of the rise and decline in numbers of viable bacteria in culture is given in Fig. 11.

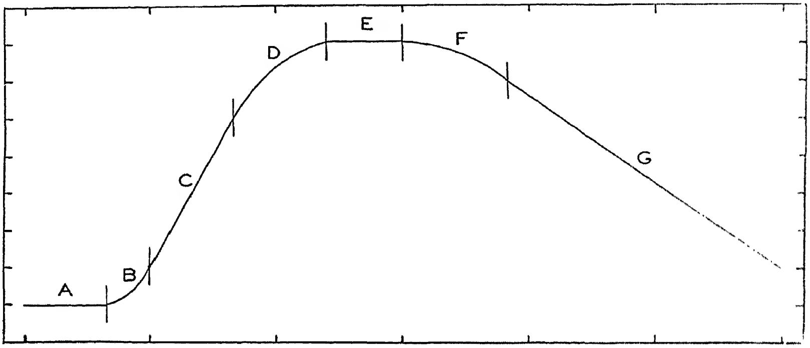


Fig. 11.—Diagrammatic representation of a bacterial growth curve; numbers of viable organisms plotted against time of incubation. *A*, initial stationary phase; *B*, positive growth acceleration phase; *C*, logarithmic growth phase; *D*, negative growth acceleration phase; *E*, maximum stationary phase; *F*, phase of accelerated death; logarithmic death phase. (After Buchanan.)

Buchanan[20] has divided this curve up into seven parts which he designates as follows:

1. *Initial Stationary Phase.*—During this phase the number of bacteria remains constant.
2. *Lag Phase or Positive Growth Acceleration Phase.*—During this phase the generation time decreases progressively until a minimum is reached.
3. *Logarithmic Growth Phase.*—In this phase the generation time remains constant and the organisms increase in numbers by geometrical progression.
4. *Negative Growth Acceleration Phase.*—The generation time progressively increases during this phase and the bacteria continue to multiply but at a decreasing rate.
5. *Maximum Stationary Phase.*—Here the numbers of viable bacteria are at a maximum, neither increasing nor decreasing.
6. *Phase of Accelerated Death.*—Following the stationary phase, the numbers of viable bacteria decrease, slowly at first but with increasing rapidity, until a relatively constant rate is attained.
7. *Logarithmic Death Phase.*—During this phase a constant rate of death is maintained. It should be noted that this rate is not maintained indefinitely for the curve tends to become asymptotic to the X axis.

Different explanations have been offered to account for the varying growth rates observed in bacterial cultures. The failure of inoculated

[20] Buchanan: Jour. Inf. Dis., 1918, *23*:109. See the review by Hinshelwood: Biol. Rev., 1944, *19*:150.

organisms to begin multiplication at the maximum rate at once has been attributed both to environmental factors and to the physiological state of the organisms. For example, it has been assumed that a partial pressure of carbon dioxide is essential to cell division for, when it is prevented from accumulating by gassing with CO_2-free air or nitrogen, growth does not take place.[21] On the other hand, the stationary and lag phases are said to be entirely eliminated by inoculation from a culture which is in the logarithmic phase of growth. Other explanations, for instance the allelocatalytic effect of Robertson,[22] have been proposed from time to time. Hershey[23] has developed mathematical equations by which the latent period in multiplication and the numbers of organisms expected at any time during the phase of rapid growth may be predicted. Since only differences in the size of the cells are postulated (see below), the satisfactory agreement of experimental data with calculated values has undoubtedly more than accidental significance. Likewise, the decreasing growth rate in the negative growth acceleration phase has been accounted for in various ways, including the rate of diffusion of oxygen into the medium, the accumulation of toxic by-products such as organic acids and ethyl alcohol, etc. It is probable that different factors are operative with different microorganisms and that each culture constitutes a special case.[24]

It is problematical whether this approach to the growth of bacterial populations has any advantages over the rationalization of parameters of fitted curves,[25] largely because the latter method has not been applied to bacteria.

The mechanisms operative in bacterial death, both in culture and in the presence of antiseptics, are uncertain. The survivor curves are logarithmic, *i.e.*, the same percentage of viable organisms is dying at any particular moment. The fact that this rate may be described by a mathematical expression which likewise describes a monomolecular chemical reaction is generally held to be nothing more than a fortuitous coincidence, and the conclusion drawn by some workers that bacterial death is a monomolecular reaction has no basis in fact.[26]

Morphologic Variation During Growth.[24]—It has been observed that both the morphology and the physiological activity of bacteria differ in different phases of the growth of a culture. As growth begins to get under way, the maximum size that a given cell reaches before fission increases somewhat, and microscopic examination shows that a large proportion of the bacteria in a culture at this stage of development are

[21] Valley: Quart. Rev. Biol., 1928, *3*:209.

[22] Robertson: *The Chemical Basis of Growth and Senescence*. J. B. Lippincott Company. Philadelphia. 1923.

[23] Hershey: Jour. Gen. Physiol., 1939, *23*:11.

[24] Henrici: *Morphologic Variation and the Rate of Growth of Bacteria*. Charles C Thomas. Springfield and Baltimore. 1928.

[25] *Cf.* Chapman: Ecol., 1928, *9*:111.

[26] Likewise, the logistic curve describes an autocatalytic chemical reaction, but this is neither evidence for the autocatalytic nature of population growth, nor does it constitute rational grounds for discarding the logistic curve as a description of population growth because "growth is not an autocatalytic process."

appreciably larger than at other times. The cells stain evenly, and there is no evidence of granular structure even in those organisms in which metachromatic granules are most readily demonstrated. The rate of respiration per cell increases, reaching a maximum at the end of the phase of accelerating growth and declining as the culture goes into the logarithmic growth phase. The increased metabolic rate in the early growth stages is, however, more apparent than real and has been shown[27] to be quantitatively related to cell size. The cell size declines also as the culture goes into the logarithmic growth phase although the individual cells remain homogeneous and take stains evenly. By the time the stationary phase is reached, the bacterial cells are uniformly smaller and, in cultures of spore-forming organisms, many are forming spores. As the viable bacterial count decreases the cells no longer stain uniformly but show a granular structure and involutionary forms appear. This succession of morphologic types, the embryonic or young cells, the mature forms and finally the senescent forms, has been designated *cytomorphosis*.[24] It is possibly analogous to a similar succession of events in the higher forms of life.

The Chemical Composition of Bacteria.—The chemical composition of bacteria is not greatly different from that of other living material. They differ in composition from species to species, and the chemical constitution of a given species is influenced to a considerable extent by the composition of the medium upon which the organisms are grown. In consequence, a precise statement of the relative amounts of the compounds and elements of which these organisms are composed is not possible; only crude approximations may be made.

Bacterial cells contain considerable quantities of water but somewhat less than the cells of the higher plants and animals. Estimates of water content vary widely, very likely owing to the difficulty of getting accurate wet weights. Although some organisms have been reported to contain as much as 90 per cent water, most bacteria show, in the hands of careful workers, 70 to 80 per cent water. In some species of bacteria an appreciable part, 17 to 28 per cent, of this is bound water.[28] The ash content, *i.e.*, the inorganic material remaining after combustion of the cells, depends to some degree, both qualitatively and quantitatively, on the inorganic content of the medium on which the organisms are grown. The values are, therefore, subject to considerable variation, and have been given as from 2 to 14 per cent of the dry weight of the cells. The ash is largely phosphoric acid, the P_2O_5 content ranging from 10 to 45 per cent of the total ash. Undoubtedly a considerable proportion of this phosphorus exists in the cell in the form of nucleic acids. Sulfur, potassium, chlorine and calcium are also present in notable amounts, together with usually smaller quantities of magnesium, iron, silicon, etc.

There is a good deal better agreement regarding the carbon content of bacteria, for most analyses give about 50 per cent of the dry weight as carbon. A part of this, of course, is present in the cell as protein, but the polysaccharide gums which make up the capsular material account for a

[27] Hershey and Bronfenbrenner: Jour. Gen. Physiol., 1938, *21*:721.
[28] *Cf.* Friedman and Henry: Jour. Bact., 1938, *36*:99.

considerable portion. Cellulose is not a common constituent of bacteria but has been identified in one organism.[29] Hemicellulose is often present, and starchlike material (granulose) is found within the cells of some species.

The total nitrogen of bacterial cells varies more widely. An estimate of 8 to 15 per cent of the dry weight is in accord with the majority of analyses. A part of the nitrogen is present in the cell as protein, but very little is known of bacterial proteins. Both albumins and globulins have been isolated, the latter much more frequently. A fraction obtained by extraction by dilute alkali and called nucleoprotein has been of immunological interest and has been studied more thoroughly than other protein constituents. It appears to be a globulin combined with nucleic acid.

A finding in keeping with the high phosphorus content of bacterial ash is the relatively large proportion of the total nitrogen that may be accounted for as nucleic acid. These organisms contain a higher proportion of nucleic acid than any other tissue except thymus, a characteristic they share with the yeasts and molds. As much as 7.1 to 11.5 per cent of the total nitrogen is purine nitrogen. Adenine, guanine, cystosine and thymine have been isolated from the tubercle bacillus, the presence of thymine and the absence of uracil suggesting the animal type of nucleic acid. Guanine has been isolated from the colon bacillus and cytosine and uracil from the cholera vibrio. Thymine is absent from the latter, suggesting in turn the plant type of nucleic acid. In addition to the nucleic acids proper (polynucleotides), their decomposition products occur in the cell, including mononucleotides, nucleosides and the free bases. The structure of bacterial nucleotides is uncertain although the predominating base is adenine. The function of adenyl pyrophosphate as a phosphate carrier in fermentation is discussed elsewhere (p. 102).

Some bacteria contain considerable quantities of fats, lipoids and waxes, *i.e.*, ether extractable material. The variation among bacteria as a group is great, analyses indicating that from 2 to 40 per cent of the dry weight may be lipoid in nature. Here, however, the variation within a species is not so great, but certain species, such as the acidfast bacteria, contain large amounts of ether extractable material. These, for the most part waxes and complex alcohols, appear to be associated with the acid-fast staining properties of these organisms.

[29] Hibbert and Barsha: Jour. Amer. Chem. Soc., 1931, 53:3907

CHAPTER 4

THE EFFECT OF PHYSICAL AND CHEMICAL AGENTS ON BACTERIA

The separation of the environmental factors, that so profoundly affect the life processes of living cells, into two groups, one designated physical and the other chemical, is undoubtedly artificial in many instances. It is often difficult, if not impossible, to differentiate the physical from the chemical; the lethal effect of a germicidal substance, for example, may be a result of a combination of both physical and chemical activity or its mode of action may lie in the borderland of surface and physical chemistry. Nevertheless, such a separation has a certain reality and is useful for purposes of exposition.

Bacteria, like all other living organisms, consist of protoplasm, a delicately balanced, heterogeneous mixture of various substances in colloidal and true solution. The disturbance of this equilibrium through, for instance, the precipitation of constituent protein, is incompatible with the continuation of the complex phenomena of life, and the cell dies. On the other hand, the environment, when favorable, not only does not destroy this equilibrium but makes possible its continuation through growth and multiplication of the organisms. Bacteria, like other organisms, are creatures of their environment; under favorable conditions they multiply rapidly and under unfavorable conditions either die or remain dormant in a viable state until another opportunity for growth presents itself. They cling to life with astonishing tenacity; samples of black mud from a salt lake were found to contain many viable bacteria after thirty-three years' storage.[1] In general, they are much more resistant to unfavorable circumstances than are most higher forms of life. A part of this resistance is, of course, a result of the ability of some bacteria to form spores which are relatively highly resistant, but the vegetative cells are considerably more resistant than are the cells of multicellular organisms.

It is not possible to differentiate sharply between these favorable and unfavorable factors. The congruity of a given environmental factor with the protoplasmic equilibrium that is life is, largely, a quantitative rather than a qualitative phenomenon. Although high temperatures destroy bacteria, a certain degree of warmth is essential to their growth. Distilled water is toxic to many microorganisms, yet multiplication takes place only in the presence of adequate amounts of moisture. Even the highly active germicidal chemicals often markedly stimulate the growth of bacteria when present in sufficiently low concentrations.

Temperature Relations.—Bacteria as a group will thrive under a relatively wide range of temperature conditions. Some of the more hardy,

[1] Rubenchik and Khait: Ann. Inst. Pasteur, 1937, *58*:446.

such as *Bacillus subtilis*, will grow throughout the range of 6° to 50° C. Others, such as many of the pathogenic forms, are able to grow over a much narrower range, and some of the more fragile organisms will grow only at body temperature, *i.e.*, 37° C., or very close to this temperature. For all these organisms, however, three temperature limits may be distinguished. There is the *minimum* or lowest temperature at which a given organism will grow, an *optimum* or temperature of most luxuriant growth, and a *maximum*, the highest temperature at which growth can take place. The position of these three points differs greatly among different species of bacteria. In general those organisms whose natural habitat is soil or water have optimum temperatures of 22° to 28° C., while those which, presumably as a result of adaptation to a parasitic mode of existence, cannot survive outside the animal body have an optimum of 37° C.

There are, however, bacteria whose optimum temperatures differ considerab y from these. Organisms have been found whose optimum temperatures are from 15° to 20° C., and they have been termed *psychrophiles* or cold-loving organisms. Others having optimum temperatures of 55° to 65° C. may be found in the soil and hot springs[2] and are called *thermophiles*. The great majority of bacteria have optimum temperatures which lie between these two extremes and, in this terminology, are designated as *mesophiles*.

The optimum temperature for given species of bacteria is generally considered to be that temperature at which the organisms grow "best." The question arises as to whether "best" refers to the rate of growth or to the maximum population attainable. It has been found[3] that temperatures optimum for growth in terms of rapidity of cell division are not always the same as those optimum for the attainment of maximum numbers of cells per unit volume. A culture grown at a temperature at which cell multiplication is most rapid will not attain as high a peak in numbers as a culture of the same organism growing more slowly at a lower temperature.[4] Other physiological activities of the cell appear to have optimum temperatures that differ from those which are optimum for multiplication. A given sugar may be fermented to a greater extent, albeit more slowly, when the culture is incubated at a temperature somewhat below that optimum for cell division. Similarly, the anthrax bacillus forms spores most abundantly at 30° to 32° C., while its optimum for vegetative multiplication is 37° C.

The continued growth of bacteria at temperatures somewhat higher than optimum may induce physiological changes of a temporary or permanent character. *Bacterium prodigiosum*, for example, fails to form its characteristic red pigment when incubated at temperatures higher than 30° C., but the change is temporary, for subcultures incubated at lower temperatures form pigment normally regardless of how many transfers have been grown at the higher temperature. The anthrax bacillus, on the other hand,

[2] Bacteria have been found in hot springs living at a temperature of 89° C. *Cf.* Setchell: Science, 1903, *17*:934.

[3] Graham-Smith: Jour. Hyg., 1920, *19*:131.

[4] See, for example, Spicer: Jour. Bact., 1940, *39*:517; Stern and Frazier: *ibid.*, 1941, *42*:479, 501.

when grown for several transfers at 42° C. loses its ability to form spores and becomes avirulent—a change that appears to be permanent. Incubation or storage of bacterial cultures at temperatures lower than optimum does not result in such qualitative physiological changes; the metabolic activities of the organisms are slowed down and at temperatures below the minimum for growth the bacteria become dormant.

The mechanisms determining the optimum, minimum and maximum temperatures of bacteria are obscure. In some cases they may be dependent upon other environmental factors. It has been shown,[5] for example, that while many of the thermophilic bacteria are able to grow only at temperatures above 50° C. when in contact with air, they are able under anaerobic conditions to grow at the ordinary incubator temperature (37° C.) or even as low as 34° C. More recent work[6] indicates that the maximum growth

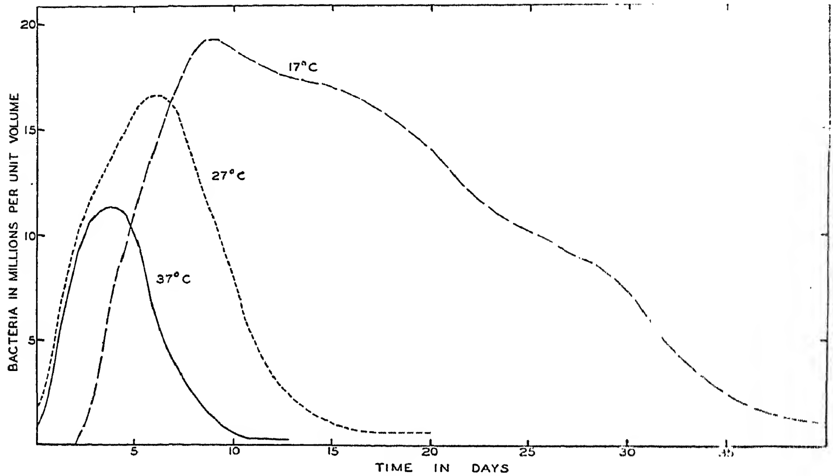


Fig. 12.—The effect of different temperatures of incubation on broth cultures of *Staphylococcus aureus.* (After Graham-Smith.)

temperatures of bacteria bear a definite relationship to the minimum temperature of destruction of respiratory enzymes.

The rate of bacterial growth and metabolic processes increases, like other chemical reactions, with rise in temperature. The temperature coefficient, *i.e.*, the quantitative effect of temperature changes, is such that these rates are roughly doubled for every ten degree rise in temperature. This effect is observed only over a relatively narrow temperature range, from the minimum to the optimum temperature of the bacterial species under consideration.

The Lethal Effects of Heat.—Bacteria are readily killed by heat, and the utilization of heat in one form or another is one of the most convenient

[5] Rabinowitsch: Ztschr. f. Hyg., 1895, *20:*154.
[6] Edwards and Rettger: Jour. Bact., 1937, *34:*489.

means of their destruction. The lethal effects of heat are markedly influenced by the amount of moisture present, and so-called *moist heat* is a much more effective killing agent than dry heat. The resistance of bacteria to moist heat differs somewhat from species to species, the pathogenic forms being in general somewhat less resistant. The resistance of a given species is influenced by two important factors, the ability of the organisms to form spores and the previous history of the culture. Spores are always much more resistant than vegetative forms. Some species when in the spore stage can withstand the temperature of boiling water for upward of sixteen hours. The vegetative forms of most bacteria, on the other hand, are killed at 55° to 58° C. by ten minutes' exposure in the presence of moisture. In general the heat tolerance of bacteria appears to be directly related to maximum growth temperatures.[7] The time and temperature of incubation influence to some extent, the heat resistance of vegetative cells. Actively growing cultures in the logarithmic phase are generally somewhat less resistant than are cells removed from cultures containing maximum numbers of viable organisms. The temperature of incubation appears to affect thermal resistance somewhat, in that cultures grown at and above the optimum temperature are more resistant than cultures grown at sub-optimal temperatures.[8] Previous treatment of both spores and vegetative cells with sublethal doses of ultraviolet light reduces their thermal resistance. However, such changes in resistance are usually of no great magnitude. Of considerably greater quantitative importance is the *p*H of the liquid in which the organisms are suspended. Variations from neutrality increase, to a marked degree, the rate at which a given temperature kills bacteria. Acid foods, such as tomatoes, are more readily preserved than foods which have a neutral reaction. Likewise, the addition of sodium carbonate to water in which surgical instruments are boiled increases the efficiency of the heat and at the same time tends to reduce rusting.

The death of an organism from heat is determined not only by the temperature reached but also by the time of exposure. The tubercle bacillus, for example, is killed by thirty minutes' exposure at 58° C., twenty minutes at 59° C. and two minutes at 65° C. One may, therefore, determine the *thermal death point* of a given bacterium by exposure to varied degrees of heat for a constant time; or, similarly, a *thermal death time* may be determined by holding the temperature constant and varying the time of exposure. Both are useful, the latter possibly somewhat more so.

The effect of heat seems to be injurious even when bacteria are not killed, since the cells that have been heated appear to require a longer period of germination. Hershey[9] has shown that exposure to sublethal heat prolongs the latent period preceding cell division without affecting the rate of regeneration of the respiratory function, with no evidence of a period of "recovery" distinct from growth. On the other hand, sublethal heating of bacterial spores often has the effect of accelerating germination.[10]

[7] *Cf.* Lamanna: Jour. Bact., 1942, *44*:29.
[8] *Cf.* Elliker and Frazier: Jour. Bact., 1938, *36*:83.
[9] Hershey: Jour. Bact., 1939, *38*:563.
[10] *Cf.* Evans and Curran: Jour. Bact., 1943, *46*:513.

The application of moist heat to the destruction of bacteria may take several forms. Sterilization by means of steam under pressure is the most efficient of these, because it makes possible temperatures higher than 100° C. in the presence of moisture. At fifteen pounds' pressure, for example, the temperature will be 121.3° C., at twenty pounds, 126.2° C., etc. As a rule, exposure to fifteen pounds' steam pressure for fifteen minutes suffices for the complete destruction of both vegetative and spore forms of bacteria, although rarely highly resistant spores may be found that are not killed by this exposure. Boiling, *i.e.*, exposure to 100° C., suffices to kill all vegetative forms of bacteria within a few minutes; but sterilization is not effected, for the spores of many of the saprophytic bacilli are not destroyed by boiling for many hours. Since very few of the pathogenic bacteria form spores, boiling contaminated water for a few minutes renders it safe for drinking purposes and the boiling of surgical instruments generally suffices to kill harmful bacteria.

Temperatures as high as 100° C. are not necessary for the destruction of vegetative forms of bacteria, since most of them are killed at 55 to 58° C. if exposed for ten to thirty minutes. In the preparation of vaccines, suspensions of bacteria are usually heated to 60° C. for thirty minutes to one hour (to allow an adequate margin of safety). The tubercle bacillus, *Brucella abortus* and other pathogenic organisms occurring in milk are killed by the process of pasteurization, *i.e.*, heating to 142° to 145° F. for thirty minutes.

Dry heat is much less effective as a germicide than moist heat. Temperatures of 160° to 170° C. must be maintained for two to three hours in order to ensure complete destruction.

The destructive effect of high temperatures on bacteria is apparently associated with the coagulation of constituent protein. The rate of heat coagulation of protein in solution closely parallels the rate of destruction of bacteria by hot water (p. 73). Furthermore, the effect of the water content of egg albumin on the temperatures necessary for coagulation is similar to the observed relative efficiency of moist and dry heat in the killing of bacteria.[11]

Cold.—Bacteria are much less sensitive to low than to high temperatures. As the minimum temperature of a given species is approached and passed, the metabolic activities of the organism become increasingly slower until a state approaching dormancy is reached. Preservation of bacterial cultures in the refrigerator is a common practice, for the rate of death of most bacteria is greatly reduced at low temperatures.[12] Some mortality results from freezing; it is thought to be due to mechanical grinding coincident with the formation of ice crystals. The evidence for this is, however, not altogether indubitable, and attempts to disrupt bac-

[11] Egg albumin in aqueous solution is coagulated at 56° C.; with 25 per cent water content at 74–80° C.; with 18 per cent water content at 80–90° C.; with 6 per cent water content at 145° C. Anhydrous egg albumin may be heated to 170° C. without coagulation.

[12] Some species of bacteria, however, are particularly sensitive to cold; gonococci and meningococci, for example, die out much more rapidly in the refrigerator than in the incubator.

terial cells by alternate freezing and thawing are not particularly successful. These organisms show a remarkable resistance to extreme cold; exposure of organisms like the typhoid and diphtheria bacilli to liquid air (−190° C.) and to liquid hydrogen (−250° C.) does not destroy their vitality. In this connection it is of interest that it has been found that muscle fibers frozen in liquid air for five hours respond to electrical stimulation on thawing.[13] Recent work[14] has indicated that the temperature at which frozen bacteria are stored significantly influences the death rate. When *Bacterium coli* was kept at −20° C. 25 per cent of the organisms were still alive after 163 days, while at −2° C. death occurred rapidly and only 4 per cent were found viable after eleven days. Death under such circumstances may possibly be a result of the denaturation of constituent protein. The viability of pathogenic bacteria in ice from contaminated sources is discussed elsewhere (p. 223).

Drying.—The vegetative forms of most bacteria are killed by drying in air, although the different species exhibit pronounced differences in their resistance. The tubercle bacillus is one of the more resistant and the cholera vibrio one of the more sensitive to drying. In general, the capsulated organisms are more resistant than the non-capsulated forms. Spores are quite resistant to drying; the spores of the anthrax bacillus, for example, will germinate after remaining in a dry condition for ten years or more. The resistance of the pathogenic forms causing disease of the upper respiratory tract is of particular interest in connection with air-borne infection (p. 201), for the length of time that a droplet remains infective is a result, primarily, of the resistance of the particular microorganism to drying.

The resistance of a given organism to drying is apparently determined to some extent by the rapidity of the drying process and the temperature at which the organisms are dried and stored. If bacteria are frozen rapidly, as with dry ice and alcohol or one of the glycols, dried from the frozen state (the lyophile process) and the container or ampoule evacuated and sealed, they remain viable with antigenicity and virulence unimpaired for months and years when stored in the refrigerator.[15] It is preferable that they be suspended in a protein-containing solution such as dilute serum before freezing; in the writer's experience broth cultures frequently do not survive. Even bacteria which are sensitive to both cold and drying may be preserved intact by this method. The process is also applicable to sera and similar materials.

Light.—Bacteria do not contain chlorophyl and, with the exception of the purple and green sulfur bacteria (p. 94), are unable to make use of radiant energy. Direct sunlight is injurious to most bacteria, and diffuse daylight exerts a hindering effect on bacterial growth and metabolic activity. This bactericidal activity is most pronounced at the ultraviolet region of the spectrum beginning at 3650 Å. The most active wave lengths appear to be 2530 and 2650 Å. In sublethal amount, however, ultraviolet

[13] Luyet and Thoennes: Compt. Rend. Soc. Biol., 1938, *207*:1256.
[14] Haines: Proc. Roy. Soc., Ser. B, 1938, *124*:451.
[15] Flosdorf and Mudd: Jour. Immunol., 1935, *29*:389.

irradiation stimulates the growth and respiration rate of microorganisms, one of the most interesting effects being an apparent initial increase in the number of colony-forming organisms. Although this invisible portion of the spectrum is by far the more actively germicidal, visible light also exhibits germicidal activity, blue being the most active and green the least. Visible light requires a much longer exposure to produce lethal effects. Sterile media exposed to sunlight for considerable periods of time are rendered unfit to support the growth of inoculated bacteria, apparently as a result of the formation and accumulation of hydrogen peroxide.

The mechanism of the action of ultraviolet light on bacteria is uncertain. Studies on the problem from the point of view of the quantum theory[16] have indicated that about four million quanta of energy are necessary to kill a single colon bacillus, and presumably the effect is a general one on the bacterial protoplasm.

The relatively weak bactericidal activity of the visible spectrum may be enhanced by the inclusion of certain fluorescent dyes in the suspending medium. This *photodynamic sensitization* is of such degree that visible light becomes almost as effective as ultraviolet light in the destruction of bacteria, protozoa and viruses. Oddly enough, gram-negative organisms appear to be more resistant to such sensitization than gram-positive ones. The mechanism of this effect is entirely unknown.

Other Radiations.[17]—The effect of a number of other radiations on bacteria has been the subject of some investigation. *Cathode rays* have been found lethal to a number of bacteria, the absorption of a single electron by a given cell being sufficient to cause death, presumably as a result of the release of large numbers of ions coincident with the absorption of the electron. Whether or not *x-rays* have a lethal effect is uncertain; some workers have found that these radiations are bactericidal while others have found no such effect. It has been observed that mutation-like changes are produced in some bacteria by *x-ray* irradiation.[18] Likewise, the evidence regarding the activity of *radium emanations* is conflicting as regards the α and β particles, although there is general agreement that the γ rays are harmless. *Short radio waves* appear to have no effect other than that resulting from heat produced. It has been thought by some that there are certain radiations, termed *mitogenetic rays*,[19] produced by actively dividing cells which stimulate the proliferation of neighboring cells. It is not established that such radiations exist.

Other Physical Agents.—*Sonic and supersonic vibrations* apparently destroy vegetative cells upon exposure for sufficient lengths of time. Audible sound waves vibrating at 8900 cycles per second have been found to reduce the numbers of viable organisms in a suspension by 99 per cent in a period of forty to sixty minutes. Ultrasonic waves of 300,000 to 1,000,000 cycles per second have been found to reduce the numbers of viable *B. coli*

[16] Wyckoff: Jour. Gen. Physiol., 1932, *15*:351.

[17] *Cf.* Review by Jordan, P., Naturwissenschaften, 1938, *26*:505.

[18] Haberman and Ellsworth: Jour. Bact., 1940, *40*:483; Lincoln and Gowen: Genetics, 1942, *27*:441.

[19] *Cf.* Gurwitsch and Gurwitsch: *Die mitogenetische Strahlung*. Berlin. 1932.

from 70,000,000 to 20,000 per cubic centimeter in fifteen minutes. The destruction is presumably purely mechanical if the effects of rising temperature have been controlled. Many attempts have been made to kill bacteria by passing an *electric current* through their suspensions. If these organisms could be killed in this manner, the sterilization of fluids such as milk would be a simple matter. It appears, however, that an electric current *per se* has no effect on the organisms, although the generation of heat and the liberation of chlorine (arising from the decomposition of chlorides) incidental to the passage of the current may kill the organisms. At *p*H's compatible with life, bacteria are negatively charged with respect to water and respond to an electric current by moving to the anode. The migration of these organisms in an electric field is termed *electrophoresis*. Studies on alterations and reversal of charge have shed some light on the mechanism of the agglutination reaction (p. 267). *Increased pressures* have little or no effect on bacteria unless the pressure is high, 5000 to 6000 atmospheres, and the exposure prolonged. Under such circumstances the organisms may be reduced in numbers. Spores are more resistant; the spores of *B. subtilis*, for example, survive 20,000 atmospheres. On the other hand, animal cells are killed by pressures of 1800 atmospheres.[20] Increased pressures of carbon dioxide show more bactericidal activity, non-spore-forming bacteria being killed after ninety minutes' exposure to a pressure of 50 atmospheres. Such a germicidal effect may possibly be a result of increased acidity. The sudden release of high carbon dioxide pressures may result in the disruption of the cells of some bacteria; the gram-negative forms are more readily broken up in this manner than are the gram-positive. Sudden release of pressures of other gases does not have this effect.

Chemical Agents.—The general subject of the effect of chemical agents on bacteria is a broad one and might reasonably be considered as including not only the germicidal chemicals but also the foodstuffs used by these organisms. It is more convenient, however, to consider the latter in terms of the effects of bacteria on their environment in a later chapter under the general head of bacterial physiology.

A certain terminology has grown up about this subject matter which requires definition. The synonymous terms *bactericidal* and *germicidal*, which have already been used, are adjectives indicating a bacteria-killing power. The term *bacteriostasis*, or the adjective *bacteriostatic*, denotes a somewhat less drastic effect. A bacteriostatic substance is one which does not kill bacteria but acts in a preservative manner by preventing their growth. An *antiseptic* is a substance which has a preservative action, possibly killing a few of the bacteria exposed to it, but in general acting predominantly in inhibitory fashion. The term *disinfectant* is applied to substances having bactericidal activity and denotes something more vigorous than does antiseptic. These terms are obviously relative, for a substance that is bactericidal in a given concentration may be only inhibitory or antiseptic in lower concentrations. Furthermore, the specificity of a certain compound for some kinds of bacteria may result in its having

[20] Basset, Macheboeuf and Wollman: Ann. Inst. Pasteur, 1937, *58*:58.

disinfectant action with regard to one species but only antiseptic action on another.

Water.—In general, bacterial cells suspended in distilled water do not survive more than a few hours, although spores will survive for many weeks. Death of the organisms results from a variety of factors one or more of which has been operative in most of the reported experiments. Water from a metal still, for example, often contains sufficient traces of metals to be toxic, and water sterilized in soda or "soft" glass contains alkali dissolved from the glass.[21] Water freshly distilled from hard glass is neutral but upon standing absorbs carbon dioxide from the air and becomes acid. The *p*H markedly influences the survival of bacteria in water, the death rate increasing on either side of *p*H 6.0, which appears to be optimum for longevity. Other factors, such as the number of bacteria suspended in a given amount of water, dissolved oxygen and accessibility to oxygen, etc., are likewise known to affect the survival of these organisms. The osmotic pressure of distilled water, which *a priori* might be thought of considerable significance in this connection, is not an important factor because bacteria are remarkably resistant to changes in osmotic pressure and are not disrupted or plasmolyzed by suspension in hypotonic or hypertonic solutions.

Acids and Alkalis.—Both strong acids and strong alkalis, *i.e.*, those that are highly dissociated, exert a marked bactericidal effect. The lethal activity of the mineral acids is associated with, and proportional to, the degree of their dissociation, but that of the organic acids appears to be an effect of the whole molecule, for the degree of dissociation is, as a rule, not great. The disinfectant action of alkalis such as sodium hydroxide is likewise proportional to the degree of dissociation. The germicidal activity of the hydroxides of the alkaline earths is, however, greater than can be accounted for on the basis of dissociation, for the metallic ion is often toxic in itself. Both acids and alkalis in too low a concentration to kill bacteria rapidly often enhance the activity of other disinfecting agents. For example, the germicidal activity of many salts is greater in the presence of acid or alkali and, as noted above, bacteria are killed much more rapidly by heat in the presence of dilute acid or alkali than at neutrality.

The relation of bacterial growth to the acidity or alkalinity of culture media has been discussed elsewhere. Concentrations of hydrogen or hydroxyl ions compatible with growth are very low, of the order of 10^{-4} to 10^{-9} mols of hydrogen ions per liter. Almost all bacteria will grow at *p*H 7.0 (1×10^{-7} mols hydrogen ions per liter) but grow best at an optimum which varies from species to species. The minimum and maximum limits between which growth takes place likewise vary widely with species. Certain organisms such as the lactobacilli and *Streptococcus lactis* are termed *aciduric* organisms because they are able to grow profusely at *p*H 4.0 or less. Perhaps the most acid-resistant organism known is *Thiobacillus thio-*

[21] It is commonly observed that water from stills in which the condenser is lined with block tin is toxic to unicellular protozoa. Nutrient solutions sterilized in soda glass containers will not support the growth of *Chilomonas paramecium* while the same solution sterilized in pyrex glass will. *Cf.* Burrows: Protoplasma, 1938, *31*:20.

oxidans. This organism accumulates sulfate in its cultures as an end product of the oxidation of sulfur and continues to grow even in the presence of N/10 sulfuric acid.[22]

Salts.—Salts have, in general, two effects on bacteria. In very low concentrations they markedly stimulate growth and at higher concentrations they become toxic. The particular concentrations at which these effects are apparent are dependent upon the degree of dissociation of the salt, the nature of the anion and the valency and molecular weight of the metallic ion. In general, the bivalent cations are more toxic than the monovalent cations and the salts of the heavier metals are more toxic than those of the lighter metals. There is, however, no precise quantitative relation in either case.

The most active of the heavy metals are mercury, silver and copper. Mercuric chloride is highly active in 0.1 per cent aqueous solution, killing both spores and vegetative cells within a short time, and the silver salts, such as silver nitrate, although somewhat less active, are still highly efficient germicides. Copper salts are still less active but are highly efficient in the destruction of algae and other chlorophyl-containing organisms. Dissociation of these salts is intimately related to their disinfectant properties. A solution of mercuric chloride in absolute alcohol has substantially no disinfecting power, but if water be added the germicidal power of the solution increases proportionately to the amount of water added. Because of the importance of ionization, a comparison of the bactericidal power of the various metallic salts on the basis of percentage solution is misleading; equimolecular solutions must be used and the ionization constants taken into consideration. The bactericidal activity of the heavy metal salts is a result of the affinity of the cations for protein material; when the constituent protein of a bacterial cell is precipitated as an insoluble proteinate, the cell dies. Other factors appear to be involved also, however. Guest and Salle[23] have observed that inorganic metallic salts which are only slightly bactericidal individually may become markedly active when mixed to produce an oxidation-reduction system.

The *oligodynamic action of metals* is possibly a result of the solution of the metal to form salts. The destruction of bacteria in contact with or in proximity to a piece of metal is the basis of some methods of disinfection of water. Water may be sterilized by allowing it to seep through a layer of silver-coated sand, and water containing colloidal silver in amounts sufficiently small to defy chemical detection, by calculation about 40 gamma per cubic centimeter, is markedly bactericidal. Such colloidal solutions are prepared by sputtering silver electrodes in water—the so-called catadyn process.[24]

The other cations are less toxic than mercury and silver, but even sodium and potassium are toxic to bacteria in sufficiently high concentrations (*ca.* 2 molar). It is of some interest that the arrangement of cations in an order of their toxicity—mercury and silver at one end and sodium and

[22] Waksman and Joffe: Jour. Bact., 1922, *7*:239.
[23] Guest and Salle: Proc. Soc. Exp. Biol. Med., 1942, *51*:272.
[24] For a discussion see Hoffmann: Arch. f. Hyg. u. Bakt., 1938, *120*:147.

potassium at the other, with others falling in order in between corresponds closely to the Hofmeister series and the lyotropic series of Freundlich, in which cations are arranged in the order of their effects on physical properties of proteins, such as coagulation, solubility, viscosity, etc. The toxicity of cations as manifested in solutions containing a single salt may often be neutralized by the presence, in the proper proportion, of another cation. This phenomenon, known as the *antagonistic effect of salts*, has led to the concept and preparation of so-called balanced solutions such as Ringer's solution, Locke's solution and others. Salts not only modify or enhance the toxic qualities of other salts but also exert similar effects on disinfectant compounds of widely different constitution. Sodium chloride, for example, markedly enhances the germicidal qualities of phenol for anthrax spores when present in sufficiently high concentrations.

The part which anions play in the growth and destruction of bacteria is less well known. Some, particularly those containing sulfur, carbon, nitrogen or oxygen, may serve as sources of these elements and of energy but in appropriate concentrations many are toxic for bacteria.[25]

Oxidizing Agents.—Other salts, such as potassium permanganate and the sodium and calcium salts of hypochlorous acid ($HOCl$), show marked bactericidal activity owing to their properties as oxidizing agents. Mol for mol, hypochlorous acid is one of the most powerful germicides known, and its calcium salt (commonly known as bleaching powder) has a wide use in the treatment of private and small municipal water supplies. Hypochlorous acid reacts with organic compounds containing an amide group with the formation of compounds known as chloramines. These compounds show strong disinfectant properties which are apparently associated with the presence of the $=NCl$ group. Two of these, chloramine-T and dichloramine-T, were used with considerable success in the disinfection of deep wounds in the last war. Similar compounds such as the chlorates and perchlorates likewise are bactericidal. The halogens, chlorine, bromine and iodine, are also potent germicides but, in contrast to the heavy metals, in inverse order to their atomic weights. Liquid chlorine is widely used in the treatment of water supplies and iodine in the form of its tincture is an efficient skin disinfectant. Bromine has been used occasionally as a disinfectant for swimming pool water.[26] Fluorine compounds are seldom used as disinfectants, although sodium fluoride is toxic to some bacteria, presumably owing to its interference with their oxidative mechanisms. Both hydrogen peroxide and ozone are bactericidal, but the former is rapidly decomposed by tissue catalase and has little penetrating power when applied to wounds and abrasions.

Organic Compounds.—More or less successful attempts have been made to utilize the germicidal activity of mercury and silver through the preparation of organic compounds of them which, while having disinfectant properties, are not markedly toxic to body tissue. These include metaphen (4-nitro-5-hydroxy-mercuriorthocresol), merthiolate (sodium

[25] For a complete discussion of the effect of salts on bacteria see Falk: Abstr. Bact., 1923, *7*:33, 87, 133.

[26] Hildesheim: Tech. Gemeindeblatt, 1936, *39*:56.

ethylmercurithiosalicylate), mercurophen (sodium oxymercuriorthonitrophenolate), mercurochrome (dibrom-oxymercurifluorescein) and a series of silver-protein compounds such as argyrol, protargol, argonin and the like. The organic compounds of mercury are generally used as skin disinfectants and, in dilute solution, as preservatives. The silver compounds find wide use in the disinfection of mucous membranes, as in the treatment of gonorrhea and eye infections. They are, however, quite ineffective against spores of the obligate anaerobes, Welch's bacillus of gaseous gangrene and the tetanus bacillus.[27]

Aside from these metal-organic compounds, the most efficient organic disinfectants are the coal tar products, phenol, or carbolic acid, and the monomethyl phenols, or cresols. Phenol is commonly used in 5 per cent aqueous solution and at this concentration destroys vegetative cells rapidly and spores somewhat more slowly. The cresols, ortho, meta and para, are not soluble to this extent but in 2.5 per cent emulsions are about three times as bactericidal as 5 per cent phenol. Crude tricresol, a distillate containing various impurities as well as all three cresols, is a more potent disinfectant than any of the cresols alone or a mixture of the three in pure form. Difficulties arising from the low solubility of tricresol may be eliminated by saponification and the end product, lysol, is likewise strongly bactericidal. The germicidal activity of the phenol derivatives may be enhanced by halogen substitution, and if the methyl group of the halogen compound is replaced with aromatic or higher aliphatic groups, toxicity for bacteria is still further increased.[28] Resorcinol, hydroxy phenol, is mildly bactericidal and one of its derivatives, hexyl resorcinol, has gained an undeserved reputation as a general disinfectant.[29]

Soaps, the sodium and potassium salts of higher fatty acids, are mildly germicidal in themselves, but probably what disinfectant action they possess may be accounted for on the basis of mechanical removal of micro organisms by emulsification of the lipoidal secretions of the skin in which many bacteria become embedded. The surgical soaps, containing a disinfectant, are not as effective germicides as might be supposed, although somewhat more bactericidal than ordinary soaps.[30] The slight bactericidal action of fatty acids is apparently attributable to the hydrogen ion.[31] During recent years a group of substances similar to soaps, the sodium alkyl sulfates (such as Drene and others), have come into widespread use. These are cationic detergents in contrast to the soaps which are anionic detergents. Some of these have been found[32] to inhibit the growth of some bacteria in relatively high concentrations (0.1 per cent), but the activity is markedly selective, gram-positive bacteria being inhibited but gram-

[27] Brewer: Jour. Amer. Med. Assn., 1939, *112*:2009.

[28] Klarmann, Shternov and Gates: Jour. Lab. Clin. Med., 1934, *19*:835; *ibid.*, *20*:40.

[29] See the review of the relation of structure of the phenols and their bactericidal properties by Suter: Chem. Rev., 1941, *28*:269.

[30] For a discussion of the bactericidal activity of soaps see Eggerth: Jour. Gen. Physiol., 1926, *10*:147; Jour. Exp. Med., 1927, *46*:671; *ibid.*, 1929, *49*:53; *ibid.*, *50*:299; *ibid.*, 1931, *53*:27.

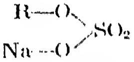
[31] Cowles: Yale Jour. Biol. & Med., 1941, *13*:571.

[32] *Cf.* Cowles: Yale Jour. Biol. & Med., 1938, *11*:33.

negative bacteria not affected. Reduced surface tension in itself has little detrimental effect on bacteria, although it might be supposed that a germicidal substance which markedly reduced the surface tension of water would be more effective as a result of concentration at the bacterium-water interface. In the case of surface active germicides it has been observed[33] that wetting action and bactericidal properties are closely cor-

$R{-}COONa$

soap



alkyl sulfate

related. Whatever the mechanism, it has been found that wetting agents apparently increase the bactericidal action of undissociated phenols.[34] The salts of quaternary ammonium bases are also cationic detergents. These have been of interest in recent years and appear promising as germicides, especially those having 12 to 16 carbon alkyl chains.[35]

Ethyl alcohol and ethyl ether, often used as skin disinfectants, are not good germicides. Their effectiveness probably lies in the solution of the lipoidal secretions of the skin and consequent mechanical removal of microorganisms. Absolute alcohol has little or no germicidal activity. The bactericidal activity of alcohol-water solutions increases with the addition of water until the solution contains approximately 50 per cent alcohol, but decreases with a further decrease in alcohol concentration. Absolute propyl and isopropyl alcohols are likewise ineffective but show activity in aqueous solution, while absolute methyl alcohol is said to be bactericidal.[36]

Dyes.[37]—The dyes are widely used in bacteriology both for staining purposes and as indicators. In addition, many of them show a marked bacteriostatic and bactericidal activity which is often specific in that it is manifested against one organism and not another. The incorporation of an appropriate dye in a medium will render it selective, *i.e.*, it will favor the growth of some species of bacteria and inhibit that of others. In general, this specificity is correlated with the gram reaction; the gram-negative organisms are, for the most part, much less sensitive to dyes than are the gram-positive species. The mechanism of this selective action is obscure.[38] The antiseptic activity of these compounds is affected by *p*H, the toxicity of the acid dyes increasing with acidity and that of the basic dyes increasing with alkalinity.

Many of the dyes such as the thiazins, oxazins and azo dyes are not particularly toxic for bacteria, dilutions of 1:1000 or less being required to inhibit growth. A number of the triphenyl methane dyes are, on the other hand, inhibitory in high dilutions. Malachite green, for example,

[33] Cowles: Yale Jour. Biol. & Med., 1940, *12*:697.
[34] Ordal and Deromedi: Jour. Bact., 1943, *45*:293.
[35] *Cf.* Rawlins, Sweet and Joslyn: (Sci. Ed.) Jour. Amer. Pharm. Assn., 1943, *32*:11; Fuller: Biochem. Jour., 1942, *36*:548.
[36] Lockemann, Bär and Totzeck: Centralbl. f. Bakt., Abt. I, Orig., 1941, *147*:1.
[37] For a more detailed discussion *cf.* Buchanan and Fulmer: *Physiology and Biochemistry of Bacteria.* Vol. II. Williams & Wilkins Company, Baltimore. 1930.
[38] Churchman: Stain Technol., 1926, *1*:27.

inhibits the growth of *B. subtilis* in dilutions of 1:4,000,000 and staphylococci in 1:1,000,000, while higher concentrations, *i.e.*, 1:30,000 to 1:40,000, are required to inhibit the colon and typhoid bacilli. Victoria green, a dichlor derivative of malachite green, is bacteriostatic to about the same degree. Brilliant green is active in even higher dilutions, inhibiting *B. subtilis* in a dilution of 1:15,000,000 and staphylococci in 1:4,000,000 and the typhoid and colon bacilli in 1:500,000.

The bacteriostatic properties of the triamino triphenyl methane dyes, the so-called rosanilins, are apparently associated with the substitution of alkyl groups in the amido side chains. Basic fuchsin, a mixture of the unsubstituted simple dyes rosanilin and pararosanilin, is relatively weakly bacteriostatic, dilutions of 1:500,000 being required to inhibit the growth of *B. subtilis*. Acid fuchsin, a mixture of various sulfonated derivatives of basic fuchsin, is likewise only weakly inhibitory and is widely used in media as an acid indicator under the name of Andrade's indicator. On the other hand, methyl violet,[39] a mixture of tetra-, penta- and hexamethyl pararosanilin, is markedly bacteriostatic and completely inhibits the growth of bacteria such as staphylococci, diphtheria bacilli and others in dilutions of 1:1,000,000 to 1:5,000,000.[40] Approximately 150 times as much dye is necessary to suppress growth of the less sensitive gram-negative bacteria such as the colon and typhoid bacilli. There appears to be a correlation of bactericidal activity and basicity of these compounds.[41] It may be noted that there has been a renewed interest in the dyes as chemotherapeutic agents in recent years.[42]

The acridine dyes, acriflavine and trypaflavine, have been of particular interest because of their therapeutic significance. The former is actively bacteriostatic in dilutions as high as 1:3,000,000 and the latter inhibitive to staphylococci and similar organisms in 1:2,000,000 and to the relatively fragile gonococcus in dilutions of 1:10,000,000 to 1:50,000,000. The gonococcus is killed by exposure to the dye in dilutions of 1:80,000 to 1:400,000 within two or three minutes.

Gaseous Disinfectants.—The use of bactericidal gases for the disinfection of rooms, dwellings and the like (fumigation or terminal disinfection) has declined markedly in recent years with no coincident increase in the prevalence of infectious disease. The commonly used gases, formaldehyde and sulfur dioxide (generated by burning flowers of sulfur), are probably not bactericidal as gases but in aqueous solution and are effective, therefore, only in the presence of adequate amounts of moisture (a relative humidity of 60 per cent or higher). Sulfur dioxide in aqueous solution (sulfurous acid) probably owes its germicidal qualities to its acidic nature. Formaldehyde, usually sold under the trade name of formalin (a

[39] Gentian violet is a more or less impure mixture of methyl violet and dextrin. Crystal violet, hexamethyl pararosanilin, is one of the constituents of methyl violet.

[40] The relative bacteriostatic efficiency of these dyes is very high. A 1:250,000 dilution of mercuric chloride is required to suppress bacterial growth completely.

[41] Albert, Rubbo and Goldacre: Nature, 1941, *147*:332.

[42] *Cf.* McIlwain: Biochem. Jour., 1941, *35*:1311; Rubbo, Albert and Maxwell: Brit. Jour. Exp. Path., 1942, *23*:69; Russell and Falconer: Lancet, 1943, *ii*:580; Browning: Brit. Med. Jour., 1943, *i*:263.

33–40 per cent solution of the gas in water), has greater penetrating power and is a more effective germicide than sulfur dioxide. Other gases such as hydrogen cyanide have little or no effect on bacteria. Although the value of terminal disinfection is open to serious question, that of disinfestation is well established and the gases, hydrocyanic acid in particular, are widely used for the destruction of rats aboard ship, etc.

Aerosols.—The use of aerosols, bactericidal compounds finely dispersed in the air, for the destruction of air-borne pathogenic bacteria has been developed in recent years, especially by Robertson and his co-workers.[43] These investigators first used propylene glycol as a vehicle for bactericidal compounds but control experiments indicated that the glycol alone (volatilized by heat) was equally effective. The glycol is relatively non-toxic and the vapor may be breathed with impunity, but bactericidal concentrations of 50 per cent or higher are attained when droplets come in contact with suspended microorganisms. The rate of destruction of air-borne bacteria is in agreement with the assumption of chance contact with glycol droplets. Other workers have used dispersed hypochlorite and ultraviolet irradiation to kill air-borne bacteria but these methods are not too promising. These results are of considerably more than ordinary significance in that they make possible the control of air-borne infection (p. 201); the respiratory diseases have hitherto spread in the human population without hindrance.

The Specificity of Disinfectants.—The marked specificity of the bacteriostatic and bactericidal activity of the dyes has been referred to above. The property of differential toxicity is not confined to these compounds alone, however, but is exhibited to some degree by many of the bactericidal chemicals. The hypochlorites, for example, while powerful germicides for most bacteria, have little effect on the tubercle bacillus. In general, the salts of the heavy metals are least specific in their action and dyes the most, with other compounds lying between these two extremes. Certain slow oxidizing agents such as potassium dichromate exert a selective bacteriostatic effect on gram-negative bacteria, and iodine is more efficacious against these microorganisms. Similarly, among the long chain aliphatic bases, the less strongly basic amines act on gram-positive bacteria for the most part, whereas the gram-negative organisms are more susceptible to the action of the stronger bases such as guanidines and quaternary amines.[44]

Although it might be supposed that the specificity of disinfectants is an undesirable quality and that a universal disinfectant would possess great advantages, it will be apparent on second thought that specific toxicity is often highly desirable. For example, a compound which is equally effective in the destruction of both bacteria and tissue cells could not be used to advantage in the disinfection of the mucous membranes.

The Chemotherapeutic Drugs.—The phenomenon of specificity also assumes great practical significance as the basis of chemotherapy of in-

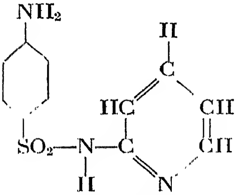
[43] Robertson *et al.:* Jour. Exp. Med., 1942, *75:*593; *ibid.*, 1943, *78:*387; Science, 1943, *97:*142.

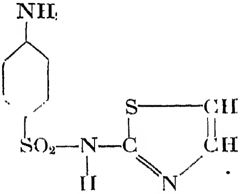
[44] Fuller: Biochem. Jour., 1942, *36:*548.

fectious disease. Ehrlich's discovery of salvarsan came as a result of a search for a "magic bullet"—a compound strongly germicidal for a given microorganism yet sufficiently non-toxic to the host that it could be injected into the tissues in effective concentrations. The discovery of chemical structures having these properties has been purely a matter of trial and error rather than rational theory, but once discovered, the active portion of the molecule may, of course, be modified to increase efficacy, decrease toxicity, etc. Successful chemotherapy has, in the past, been confined to certain spirochetoses and protozoan infections, the compounds showing activity including the arsenicals, compounds of antimony and of bismuth, certain dyes such as trypan red and the flavines, synthetic compounds such as atabrine and plasmochin, and certain naturally occurring substances such as quinine. Until relatively recently the bacterial diseases appeared to be, for all practical purposes, resistant to chemotherapy.

With the observation that the azo dye prontosil had marked chemotherapeutic activity in streptococcus infections, and the discovery of the active portion of the molecule, *p*-aminobenzene sulfonamide (sulfanilamide), a new series of compounds, known as sulfonamides, was found to


sulfanilamide


sulfapyridine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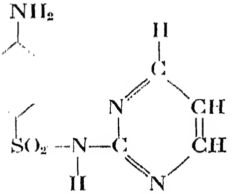
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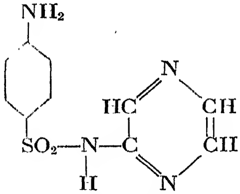
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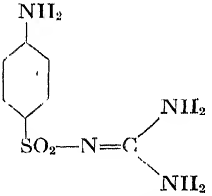

sulfaguanidine

be highly efficacious in the treatment of many bacterial infections. Many derivatives of *p*-aminobenzene sulfonamide have been prepared, radicals being attached to the nitrogen of the sulfonamide group, which show different solubilities, degrees of ionization, toxicity, rates of absorption and excretion, associated with differences in chemotherapeutic efficacy.[45]

These compounds are bacteriostatic rather than bactericidal and appear to function *in vivo* by suppressing bacterial multiplication, the invading microorganisms being destroyed by the body defenses, notably through phagocytosis by the cells of the macrophage system (p. 291). They do not

[45] *Cf.* Bell and Robin: Jour. Amer. Chem. Soc., 1942, *64*:2905.

affect the bacterial toxins to a significant degree.[46] The mechanism of growth inhibition has been of particular interest because its study has allowed the development of the first rational theory of chemotherapeutic activity.[47]

The Woods-Fildes Theory.—It was observed by Woods[48] that the bacteriostatic action of the parent compound, sulfanilamide, is specifically neutralized by *p*-aminobenzoic acid. The latter is required as a growth accessory substance (p. 121) by a few bacteria, *Clostridium acetobutylicum*, *Acetobacter suboxydans*, and possibly certain strains of the diphtheria bacillus; presumably it plays an essential part in the physiological economy of most bacteria, but the great majority are able to synthesize it and therefore do not require that it be supplied preformed. The close similarity in structure of *p*-aminobenzoic acid and *p*-aminobenzene sulfonamide will be obvious from the accompanying structural formulae. It was suggested by Woods and by Fildes[49] that the action of sulfonamides on bacteria is the result of a competition of these compounds with an essential bacterial metabolite, *p*-aminobenzoic acid. This *hypothesis of competitive inhibition* is known as the Woods-Fildes theory.

This explanation is now supported by an impressive amount of experimental evidence and it appears highly probable that it is substantially correct. For example, the bacteriostatic action of a wide variety of derivatives of sulfanilamide is nullified by *p*-aminobenzoic acid and related compounds which will give it on hydrolysis. The relationship between the antagonist and the drug is a quantitative one; the ratios usually reported vary from 1:1000 to 1:26,000 but are considerably nearer unity if the presence of antagonist in the test medium and the ionic concentration of the drug be taken into consideration.[50]

It may be noted that, as a corollary, specimens such as blood, urine or other body fluids taken from an individual undergoing sulfonamide therapy may contain sufficient amounts of the drug to inhibit bacterial growth when they are cultured. The inhibiting effect may be avoided by including *p*-aminobenzoic acid in the medium; a concentration of 5 mg. per 100 cc. is ample.[51]

The fundamental significance of this theory, however, lies in the fact that it can be generalized, and predicted antagonisms to other growth accessory substances tested experimentally. This has been done, especially by McIlwain. He has prepared sulfonic acid and sulfonamide analogues of nicotinic acid[52] and pantothenic acid[53] by substitution of $-SO_3H$ or $-SO_2NH_2$ for a carboxyl group in the essential substance, and shown them

[46] That is to say, there is apparently no clinically important effect; however, *cf.* Carpenter and Barbour: Proc. Soc. Exp. Biol. Med., 1939, *41*:354; Hutner and Zahl: Science, 1942, *96*:563.

[47] See the critical discussion by Wooley: Science, 1944, *100*:579. McIlwain: Biol Rev., 1944, *19*:135. Henry: Bact. Rev., 1943, *7*:175.

[48] Woods: Brit. Jour. Exp. Path., 1940, *21*:74.

[49] Fildes: Lancet, 1940, *i*:955.

[50] *Cf.* Fox and Rose: Proc. Soc. Exp. Biol. Med., 1942, *50*:142.

[51] Janeway: Jour. Amer. Med. Assn., 1941, *116*:941.

[52] McIlwain: Brit. Jour. Exp. Path., 1940, *21*:136.

[53] *Ibid.*, 1942, *23*:95.

to be specifically antagonistic and inhibitory. Wooley and White[54] and Wyss[55] have obtained similar results with pyrithiamin, the pyridine analogue of thiamin. McIlwain[56] has also carried out the reverse type of experiment. Iodinin, the di-N-oxide of a dihydroxyphenazine, is a pigment produced by *Chromobacterium iodinum* which inhibits the growth of certain bacteria. This growth inhibitory effect could be nullified by certain anthraquinones and naphthaquinones, from which he inferred that quinnones play some part in bacterial metabolism, possibly in the transport of hydrogen. The antibacterial effects of quinones have been studied by others also.[57] As yet none of these substances has been found to function as an effective chemotherapeutic agent.

It is of considerable interest that the generalization seems to remain valid when carried over to higher organisms. Thus, symptoms of vitamin deficiency may be produced in experimental animals by feeding an analogue of the vitamin, viz., pyrithiamin, glucoascorbic acid, isoriboflavin and the phenazine analogue of riboflavin, and the analogue of nicotinic acid, β-acetyl pyridine.

While it is probably by no means the whole story, the hypothesis of competitive inhibition seems to be firmly established, at least as a provisional theory. Attacked by some, it has been ably developed and defended by McIlwain.[58]

Another group of bacteriostatic substances, some of which are effective chemotherapeutic agents, are produced by microorganisms and are termed antibiotic substances. These are discussed elsewhere (p. 132).

Factors Influencing Disinfection.—The process of disinfection or bacterial death is often in part, at least, a chemical reaction and is, therefore, subject to a variety of influences which affect the velocity of such reactions. The most important of these influences is the concentration of the reacting substances, *i.e.*, the concentration of disinfectant and the numbers of bacteria present. The *effective* concentration of disinfectant is, in turn, dependent upon two other factors, first, the presence of moisture, which makes possible coagulation by heat, ionization of the bactericidal salts, and acts as a solvent and suspending medium in which there may be intimate contact between the disinfectant and the microorganism; and, second, the presence of extraneous organic matter. Many chemical disinfectants act through a combination with the protein of the cell and, if extraneous organic matter is present, will, of course, react with this inert material, thereby reducing the effective concentration. Disinfectants vary widely in the degree to which their bactericidal properties are affected by organic matter. The salts of the heavy metals are rapidly precipitated by organic material, while compounds such as phenol and the cresols are only slightly affected. The rate of destruction by heat is also affected by the presence of organic matter—organisms embedded in a mass of fecal ma-

[54] Wooley and White: Jour. Exp. Med., 1943, *78*:489.
[55] Wyss: Jour. Bact., 1943, *46*:483.
[56] McIlwain: Biochem. Jour., 1943, *37*:265.
[57] Armstrong, Spink and Kahnke: Proc. Soc. Exp. Biol. Med., 1943, *53*:230.
[58] McIlwain: Lancet, 1942, *ii*:412; Nature, 1943, *151:* 270; Lancet, 1943, *ii*:449.

terial, for example, are protected from heat for a short time. The process of disinfection by germicides or by heat is influenced by temperature, the velocity of the reaction increasing with rise in temperature. The *p*H likewise influences the rate of bacterial destruction not only by heat but by many chemical compounds, the velocity, in general, being least at neutrality and increasing with increase in acidity or alkalinity. A number of other factors such as the presence of salts, etc., affect the rate of disinfection but generally not sufficiently to be of practical importance.

From the practical point of view, the time of exposure of bacteria to a given disinfectant is of considerable significance and, of course, bears an inverse relation to the rapidity of killing. The time allowed for the destruction of bacteria is determined not only by the factors discussed above but also by the kind of bacteria that are to be killed. In certain cases the specificity of a disinfectant may be so marked that it must be taken into consideration. For example, the relative atoxicity of hypochlorite for the tubercle bacillus referred to above precludes its use in the disinfection of tuberculous sputum. Bacterial spores are much more resistant to heat and chemical disinfectants than are the vegetative cells, and considerably more time must be allowed for their destruction. The vegetative cells of some bacteria may be somewhat more resistant than those of others, but, for the most part, such differences are too small to be of practical significance.

The Dynamics of Disinfection.—Quantitative studies on the rate at which bacteria are killed by lethal agents have indicated that in many instances the organisms die at a logarithmic rate, *i.e.*, if the logarithms of the numbers of viable organisms are plotted against time, the points tend to fall on a straight line. This phenomenon has been observed in the death of both spores and vegetative cells under the influence of chemical disinfectants or moist heat and also occurs in the death of bacteria in old cultures. The velocity of the reaction, the slope of such a line, depends upon the concentration and kind of disinfectant, the nature of the organisms—whether spores or vegetative cells—and other factors which influence the process of disinfection. This logarithmic rate likewise describes the course of a monomolecular reaction, and this fact has led some to conclude that bacterial death is a monomolecular chemical reaction. Although the killing of bacteria by some disinfectants is undoubtedly a chemical process, as for example the precipitation of constituent protein as proteinates of heavy metals, the evidence does not justify the conclusion that the reaction is monomolecular. The fallacy is the old one of confusion of correlation and causality under the slightly different guise of description and mechanism. Experimental evidence has indicated that while such semilogarithmic plots may often be fitted with a straight line, others are best fitted by sigmoid curves, the death rate being more rapid in the beginning in some cases and more rapid at the end in others or even highly irregular. Some workers[59] have concluded that one of the more important determining factors is a graded biological resistance of the cells in the bacterial population, an explanation that is supported by general bi-

[59] Knaysi: Jour. Inf. Dis., 1930, *47*:293.

ological considerations. Undoubtedly the phenomenon of bacterial death is a complex of interrelated factors whose admixture with the mechanics of the mass law results in the parameters determined by mathematical means. Of practical importance, however, is the fact that in disinfection by chemicals and by heat there is a minority of cells, possibly more resistant, that survive long after the majority have perished and that must be destroyed in order to obtain complete sterilization.

The Standardization of Disinfectants.—The relative bactericidal efficiency of the chemical disinfectants is a point of considerable practical importance. The value of a quantitative method for the determination of the killing power of germicides was recognized early in the development of bacteriology, and experimental investigation led to the establishing by Rideal and Walker[60] of a standardized technique which made

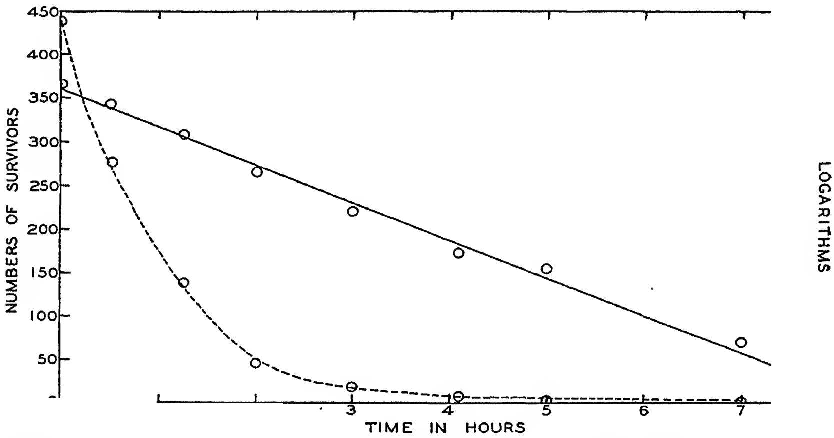


Fig. 13.—Death rate of anthrax spores treated with 5 per cent phenol. The dotted line is the arithmetic plot and the solid line the logarithmic plot. The negative logarithm was obtained by taking samples of several times the unit volume. (After Chick: Jour. Hyg., 1908, *8*:92.)

possible the determination of the bactericidal power of a given chemical compound relative to that of phenol. The numerical value so determined is termed the *phenol coefficient* and is presumed to indicate whether, and to approximately what extent, the unknown is a better or poorer germicide than phenol. Later methods of standardization have grown out of this procedure. In the method proposed by Reddish[61] the phenol coefficient of a disinfectant used against the typhoid bacillus is calculated as follows:

Divide the greatest dilution of the disinfectant capable of killing *Bact. typhosum* in ten minutes but not in five minutes by the phenol dilution which should do this and divide these figures one into another. In order not to convey a false idea of the accuracy of the method the coefficient is calculated to the nearest 0.1 point if under 1.,0 to the nearest 0.2

[60] Rideal and Walker: Jour. Roy. San. Inst., 1903, *24*:424.

[61] Reddish: *cf.* Jordan and Falk: *The Newer Knowledge of Bacteriology and Immunology.* University of Chicago Press. 1928. P. 301.

point if between 1 and 5.0, to the nearest 0.5 point if between 5 and 10, and to the nearest 1.0 point if between 10 and 20. For example, if results are read as follows:

DISINFECTANT

Dilution	Five Minutes	Ten Minutes	Fifteen Minutes
1–300	0	0	0
1–350	+	0	0
1–400	+	+	+
1–450	+	+	+

PHENOL

Dilution	Five Minutes	Ten Minutes	Fifteen Minutes
1–90	+	0	0
1–100	+	+	+

$350 \div 90 = 3.89$
Coefficient is 3.9

If the reading is as follows:

DISINFECTANT

Dilution	Five Minutes	Ten Minutes	Fifteen Minutes
1–30	0	0	0
1–35	0	0	0
1–40	+	+	+
1–45	+	+	+

PHENOL

Dilution	Five Minutes	Ten Minutes	Fifteen Minutes
1–90	0	0	0
1–100	+	+	0

then estimate the dilution of the disinfectant killing in ten but not in five minutes as 1–37.5 and the phenol as 1–95 ($37.5 \div 95 = 0.395$ or 0.4) giving a coefficient of 0.4.

The effect of extraneous organic matter on the bactericidal power of a disinfectant is commonly taken into consideration by carrying out the test with and without added organic matter. Three per cent of dried fecal matter or dried yeast may be added to the bacterial suspension or the organisms may be suspended in 50 per cent serum. It is important to recognize that bacteria differ considerably in their resistance to phenol, staphylococci, for example, being much more resistant than the typhoid bacillus, so that in strict accuracy it is necessary to specify "typhoid phenol coefficient," "pneumococcus phenol coefficient," etc.[62]

[62] For the phenol coefficient method designated as official by the Food and Drug Administration see United States Department of Agriculture Circular No. 198. 1931.

BACTERICIDAL ACTIVITY OF DISINFECTANTS*

Compound	Type	Phenol Coefficients†						Skin Sterilization‡		
		Staph. aureus		Strep. viridans		Bact. coli		Compound	Number of skin grafts	Per cent sterile
		Water	50% serum	Water	50% serum	Water	50% serum			
Merphenyl nitrate (1:1500 aqueous)	R§ A¶	0.409 614	0.133 200	0.250 375	Not done	0.272 408	0.044 66	Merphenyl nitrate tincture 1:1500	100	0
Merthiolate (1:1000 aqueous)	R A	0.188 188	0.077 77	0.031 31	Not done	0.043 43	0.03 30	Merthiolate tincture 1:1000	30	6
Metaphen (1:500 aqueous). .	R A	0.375 687	0.155 77.5	0.80 400	Not done	1.26 630	0.07 35	Metaphen tincture 1:200	50	80
Mercurochrome (1:50 aqueous).	R A	0.040 2.0	0.022 22	0.025 1.25	Not done	0.040 2.0	0.02 20	Mercurochrome tincture 1:50	40	10
Hexylresorcinol (1:1000 aqueous).	R A	0.063 63	0.022 22	0.062 62	Not done	0.043 43	0.02 20	Phenol 2% aqueous	30	0
Mercuric chloride (1:1000 aqueous).	R A	0.175 175	0.020 20	0.125 125	Not done	0.150 150	0.020 20	Alcohol acetone solution	30	0
Iodine, tincture (7% in alcohol-KI solution).	R A	20 286	0.355 5	8 112	Not done	10 143	0.35 5	Iodine, tincture 7%	40	8 0

* Selected from the data of Clampit, Campbell and Reames of the University of Chicago.
† Determined on basis of killing in five but not in ten minutes.
‡ The disinfectant is applied to an area of shaven skin, a piece of which is then excised and cultured.
§ Relative phenol coefficient determined by dilution of the disinfectant as supplied (concentration in parentheses).
¶ Absolute phenol coefficient based on the pure compound.

4

It is open to question as to whether compounds whose structure and chemical and germicidal activity differ greatly from that of phenol may legitimately be compared with this standard disinfectant. In recent years some workers have departed from the standard procedures, altering them to suit individual requirements. The phenol coefficient of an alcoholic solution of a compound insoluble in water obviously has little value, as has that determined on an acid or alkaline solution of a compound only slightly soluble at neutrality.[63] The simple statement that a given germicide has such and such a phenol coefficient has, then, little meaning.

Even assuming, however, that the standard phenol coefficient procedure be rigidly adhered to, the test itself is open to serious criticism. The end-point to be determined is, clearly, sterility—all the test organisms must be destroyed. The quantitative studies discussed above have shown that although destruction may proceed at a regular and rapid rate for a time, the asymptotic tendency of the survival curve indicates that sterility or complete destruction is not a desirable end-point in that it is one that is difficult to determine with a reasonable degree of accuracy. The practical consequences of the use of this end-point appear in the form of aberrant results when the time variable is altered. If the end-point be taken as sterility in two and one-half minutes as in the original Rideal-Walker method, the phenol coefficient of $HgCl_2$ is somewhat more than 2, but if the time taken is thirty minutes as in the Chick-Martin modification, $HgCl_2$ has a phenol coefficient of 550.[64] It would appear that a determination of the reaction velocity constant of the time-survivor curve would yield much more dependable results.

Furthermore, the phenol coefficient test does not take into consideration the temperature coefficient of the disinfectant under examination, which differs with the test organism,[65] nor does it measure the effects of changes in concentration. A rise in temperature increases the activity of phenol and similar compounds much more than that of the salts of heavy metals; doubling the concentration of phenol increases its bactericidal activity approximately 64 times, while a similar increase in the concentration of $HgCl_2$ only roughly doubles its activity.

It is generally agreed that the rate of killing, *i.e.*, the reaction velocity constant, is a much more accurate measure than any value whose derivation depends upon a sterility end-point. The relation between concentration of disinfectant and time required for killing is an exponential one and the reaction velocity constant, k, is given by the expression:

$$k = C^n t$$

when C is the concentration, n a constant characteristic for each disinfectant, and t is time. The temperature coefficient k may, of course, be determined experimentally for each disinfectant. It will be clear that this kind of characterization of the bactericidal activity of a given compound

[63] For a discussion of the limitations of the phenol coefficient see Reddish: Ind. Eng. Chem., 1937, *29*:1044.

[64] Chick: Jour. Hyg., 1908, *8*:92; *ibid.*, 1910, *10*:237; *ibid.*, 1912, *12*:414.

[65] *Cf.* Tilley: Jour. Bact., 1942, *43*:521.

is much more informative than the usual type of phenol coefficient, no matter how precisely the conditions of the test are defined. It is not, however, at all generally used for routine work.[66]

The practical value of a disinfectant is not always indicated by tests made under the controlled conditions of the laboratory. Hydrogen peroxide, for example, may give quite respectable phenol coefficients but, when applied to an abrasion, is so rapidly decomposed under the influence of tissue catalase that its germicidal powers are almost immediately exhausted.

On the other hand, a given disinfectant may be so highly toxic for tissue cells that it has no practical value. Salle and his associates[67] have proposed a "toxicity index" which takes into consideration not only the germicidal activity of a compound but its toxicity for tissue as well. For example, Witlin[68] determined the concentration of bactericidal substances lethal for the chick embryo and calculated a toxicity index by using the concentration of the bactericide in grams per cubic centimeter killing the test organism, *Staphylococcus aureus*, in ten minutes but not in five as the numerator, and the chick embryo MLD in grams for the denominator. Representative values were: phenol (1:20)—1.18; $HgCl_2$ (1:1000)—0.62; tincture of iodine—0.044; sodium hypochlorite—1.39; mercurochrome (1:50)—13.3; and values ranging as high as 9.1 for various organic mercurials. Such toxicity indices are of considerable practical importance in the case of skin disinfectants. There is as yet no standard method for their testing.[69]

The whole question of the evaluation of disinfectants is beset with many difficulties, both theoretical and practical, and to date remains an open one to which no entirely satisfactory answer has been supplied.

[66] For applications of this approach see, for example, Withell: Jour. Hyg., 1942, *42*:124, 339; Irwin: *ibid.*, 1942, *42*:328.

[67] Salle, McOmie and Schechmeister: Jour. Bact., 1939, *37*:639.

[68] Witlin: Proc. Soc. Exp. Biol. Med., 1942, *49*:27.

[69] *Cf.* Report of the Council on Pharmacy and Chemistry: Jour. Amer. Med. Assn., 1943, *121*:593.

CHAPTER 5

BACTERIAL PHYSIOLOGY[1]

The biochemical changes brought about through the activities of bacteria are by far the most obvious manifestations of their existence. The long-known but little understood phenomena of fermentation, putrefaction and decay, soil fertility and infectious diseases of man and lower animals are, in essence, no more than the exhibition of one or more of the many facets of the biochemical potentiality of these microorganisms. The oxidation of ammonia by the nitrifying bacteria, the decomposition of carbohydrates to alcohols and acids and the production of substances toxic to higher animals are a part of the normal activities of this heterogeneous group of living organisms. The elucidation of many of these phenomena has not only made possible a partial control of a not inconsiderable portion of man's environment but has provided an insight into the life processes of these organisms, organisms structurally so simple that morphology cannot carry us far in their study.

The term bacterial physiology, or, sometimes, bacterial metabolism, is a broad one which is generally assumed to include the entire sequence of events taking place in a bacterial culture. Since the small size of the individual bacterial cell precludes the utilization of many of the physiological techniques adapted to the multicellular, differentiated organism, the physiology of bacteria becomes, for all practical purposes, a biochemical physiology. The very magnitude of the biochemical changes brought about by these organisms tends to reinforce a belief in their relative importance; bacteria have, for example, been shown to consume forty to sixty times as much energy as man in terms of calories per gram of body nitrogen.[2] The obvious significance of physiological activity is so readily apparent that it has tended to obscure to many the undoubted importance of the environmental factors that influence the functioning of the living cell. It is to be borne in mind, therefore, that although bacterial physiology is a branch of general cellular physiology, it must be regarded as a special case which, in many instances, goes considerably further afield into other fundamental aspects of biology than the present concept of cellular physiology is generally assumed to do.

[1] One of the best discussions of bacterial physiology may be found in Waksman: *Principles of Soil Microbiology.* Williams & Wilkins Company. Baltimore. 1st ed., 1927; 2nd ed., 1932. For a complete survey of the literature up to 1930 see Buchanan and Fulmer: *Physiology and Biochemistry of Bacteria.* In three volumes. Williams & Wilkins Company. Baltimore. 1930. For recent literature see Stephenson: *Bacterial Metabolism.* Longmans, Green and Co. London. 2nd ed., 1939. Recent reviews may be found in the several volumes of *Annual Review of Biochemistry* and reference may be made to that of van Niel: Bact. Rev., 1944, *8*:225.

[2] Rubner: *Kraft und Stoff im Haushalte der Natur.* Leipzig. 1909.

Morphologically bacteria are simple organisms and are often regarded as a primitive type of cell. These organisms are not simple physiologically, however, many of them oxidizing glucose, for example, by mechanisms very similar to and often more complex than those used by higher animals. Others, such as the nitrifying bacteria, whose respiratory processes consist of but a single simple oxidation, synthesize all the components of protoplasm from inorganic compounds of carbon and nitrogen—a more complex process than that of joining together preformed amino acids. Since these organisms metabolize in these and other highly complex ways, it is difficult to regard them as physiologically simple. There is, however, some reason to think that bacteria are primitive cells. The view that the pathogenic organisms are degenerate forms cannot be taken to imply that the free-living forms are likewise degenerate with respect to other living cells, for such a postulate entails the assumption that the physiological capabilities of these organisms are acquired characters, and is not in keeping with current knowledge of biology. The multiplicity of reactions which bacteria are able to bring about suggests that, in these organisms, the original capabilities of protoplasm have not been lost to the extent that they have been by the differentiated cells of higher organisms. The animal body, for example, has no mechanism for the breakdown of cellulose, yet bacteria having the ability to hydrolyze this substance are universally distributed in the soil. Bacteria capable of breaking down a particular organic compound have been isolated since the early days of bacteriology by the relatively simple expedient of inoculating a highly specific medium containing the substance under consideration as the only source of energy with a complex mixture of organisms such as exist in samples of soil. It is probable that by this method a bacterium could be found which would oxidize almost any organic compound. No group of higher organisms shows such a high degree of physiological flexibility. The wide range of carbon compounds that a single bacterial species may oxidize and the multiplicity of end products of the fermentation of a single carbohydrate likewise point toward such a flexibility. The simplicity and variability of the nutritive requirements of many bacteria also suggest a physiologically primitive cell.

The physiological economy of the cell is dynamic rather than static, and life is compatible only with a state of greater or lesser, but nevertheless continuous, physiological activity. This activity may be approximately separated into two phases, the exothermic, oxidative, energy-yielding reactions, and the endothermic reduction reactions of synthesis. The former make possible a continuous, regulated supply of energy to the cell, while the latter channel this energy, or a part of it, into the synthesis of cell substance. The living cell may, therefore, be regarded as a machine for the manufacture of protoplasm, operating with a greater or lesser degree of efficiency, whose energy is supplied by the complex process of respiration. This oxidation-reduction process is not complete within itself, *i.e.*, the machine is not 100 per cent efficient, and the respiratory oxidations are not precisely balanced by the reductions of synthesis. Other substances must, therefore, be reduced to balance the excess of oxidation reactions

and this is carried out through a series of reactions leading to the eventual reduction of atmospheric oxygen. The separation of the processes of respiration and synthesis cannot be complete, of course, for in many instances the products of the respiratory process are assimilated by the cell and utilized for synthetic purposes; in fact in very many instances a preliminary decomposition is necessary before assimilation can occur.

A reasonable amount of information is available with respect to respiration, regarding both the catalysis of the reactions and the intermediary metabolism of the substrates, *i.e.*, the successive reactions involved. On the other hand, the mechanisms of synthesis of the components of protoplasm are almost completely unknown, not only for the bacteria but for other organisms as well. Elucidation of the nutritive requirements of bacteria may, perhaps, be regarded as definition of the precursors of protoplasm in the catalytic system that is the microorganism under consideration; it may equally well be regarded as definition of the limits of the synthetic abilities of the organism.

RESPIRATION

The great majority of bacteria are said to be *chemosynthetic*, *i.e.*, their metabolism is animal-like in that they obtain energy stored in complex molecular configurations by the oxidation of organic compounds. With the single exception of the purple and green sulfur bacteria, none of these organisms contains chlorophyl or similar substances which would make possible the utilization of radiant energy.

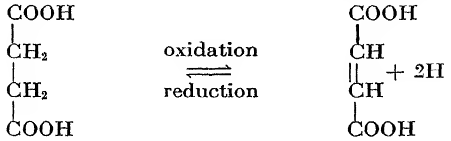
It has been shown by Novy and his associates[3] that most bacteria respire in the original sense of the term; they consume molecular oxygen and give off carbon dioxide just as do higher forms of animal life. Other workers have used the Warburg technique in the study of bacterial respiration, and it has been found possible to calculate respiratory quotients with a considerable degree of accuracy. Some bacteria, however, do not respire in this way but are able to grow in the complete absence of molecular oxygen. This discovery, made by Pasteur in 1861, was one of the landmarks of biochemical physiology, and the phenomenon of "life without air," or anaerobic growth, has since been found to occur among organisms other than bacteria, including the higher forms of life.

Anaerobic Oxidations.—Although the phenomenon of anaerobic growth appeared incredible at first, subsequent experimental work has provided a simple and adequate explanation. The oxidations brought about by living cells are, of course, coupled reactions in which a corresponding reduction takes place simultaneously. Molecular oxygen is the substance commonly reduced by cells respiring under aerobic conditions. If, however, a cell is able to reduce substances other than oxygen, the energy-yielding oxidations may proceed in the complete absence of air. Some organisms are able to grow anaerobically in the presence of nitrate, reducing it to nitrite.

In most cases, however, anaerobic oxidation-reduction reactions consist of hydrogen transfer, oxidation being the loss of one or more atoms of

[3] Novy *et al.*: Jour. Inf. Dis., 1925, *36*:109, 168, 245, 343.

hydrogen from the molecule of substrate and reduction a corresponding addition. Thus in the case of succinic and fumaric acids:



Compounds which give up hydrogen are known as *hydrogen donators* and those which receive it as *hydrogen acceptors*. Some compounds such as dyes and naturally occurring catalysts (see below), may either accept hydrogen in the oxidized state or donate it in the reduced state, the reaction being reversible. Such substances may, therefore, act as *hydrogen carriers*.

The Thunberg Technique.—These anaerobic oxidations, catalyzed by the dehydrogenases, are conveniently studied by the use of an oxidation-reduction indicator such as a dye. Methylene blue is commonly used for this purpose since it is blue in the oxidized state and colorless when reduced. The reaction must be carried out in an appropriate vessel from which air can be excluded, since leuco-methylene blue oxidizes rapidly in the presence of oxygen. This type of experiment has been developed by Thunberg[4] in particular and is known as the Thunberg technique. For example, in the study of the bacterial dehydrogenases a suspension of washed bacteria is mixed with a solution of substrate, such as glucose or an organic acid, and methylene blue in a Thunberg tube, and the tube evacuated and incubated in a water bath. With a reasonably heavy suspension of typhoid bacilli in the presence of glucose, for instance, methylene blue is reduced in a few minutes. Some standard may be taken as a reference point, such as the time of reduction of a particular concentration of a given substrate and a specified density of bacterial suspension. Suppose this standard reduction time is given an arbitrary value such as 100, then other reduction times can be expressed as one-half, one-tenth, twice or whatever it may be.

The rate of methylene blue reduction is used much more crudely as an indicator of bacterial activity in milk, and is a fairly reliable index of degree of bacterial contamination (p. 236).

The anaerobic oxidation-reduction need not necessarily involve two kinds of molecules. In that type of decomposition known as intramolecular oxidation the molecule is broken down in such a way that one portion is oxidized while another is reduced. The coupled reaction may also take place in the presence of only one kind of substrate with the oxidation of one molecule and the reduction of another; this type of decomposition is termed *dismutation* and often occurs in the bacterial metabolism of carbohydrates.

During anaerobic growth bacteria are apparently much more active biochemically than during growth in the presence of oxygen. The marked proteolytic activity of many of the anaerobic bacteria is a matter of com-

[4] Thunberg: Quart. Rev. Biol., 1930, 5:318.

mon knowledge. This increased activity is, however, more apparent than real in most cases. The most efficient oxidations, from the energy-yielding point of view, are those in which oxygen is reduced to water and carbon is oxidized to carbon dioxide. The anaerobic oxidations result in the production of reduced substances such as methane, relatively complex amines, partially oxidized substances such as organic acids, etc., and are not nearly so efficient in the production of energy as the aerobic oxidations. Consequently, much more original material must be partially oxidized to make available a given quantity of energy than would be necessary if complete oxidation, *i.e.*, to carbon dioxide and water, took place.

Respiratory Enzymes.[5]—The catalysis of the respiratory process appears to be essentially the same in all types of cells; at least this is generally assumed to be true at the present time. The respiratory enzymes make up a "system" of catalysts which function in the transport of hydrogen from the reaction mixture, *i.e.*, make possible the oxidation of metabolites, to the inexhaustible reservoir of reducible material, the oxygen of the atmosphere. They consist of a protein carrier coupled with a functional prosthetic group which is reversibly oxidized and reduced, thus making it possible for them to act as hydrogen carriers. The prosthetic groups, or portions of them, are in part members of the vitamin B complex, and are required by certain bacteria as well as higher animals (p. 123). This transport takes place in an orderly manner in that the hydrogen is passed from one to another by a series of oxidation-reduction reactions in a way analogous to the functioning of a "bucket brigade." These enzymes are:

(1) The *dehydrogenases*, which activate the hydrogen of metabolites and act as intermediaries between them and the coenzymes. (A very few, notably xanthine oxidase, are able to react directly with oxygen.) Their nature is not known but their activity is sensitive to narcotics and not affected by cyanide; they may be further separated from one another on the basis of susceptibility to selective poisons such as toluene. They reduce dyes and coenzymes.

(2) The *coenzymes*, including:

(a) *Coenzyme I* (cozymase) or diphosphopyridine nucleotide (DPN), and *coenzyme II* or triphosphopyridine nucleotide (TPN), which contain nicotinamide, the pyridine ring accepting hydrogen. They are reduced by the coenzyme-linked dehydrogenases and oxidized by flavoprotein enzymes.

(b) *Cocarboxylase* or thiamin phosphate, which catalyzes the decarboxylation of pyruvic acid to acetaldehyde in the alcoholic fermentation.

(3) *Flavoprotein* or "yellow enzyme." The prosthetic group is an alloxazine derivative, riboflavin (phosphate), and reversible oxidation-reduction occurs by virtue of two of the nitrogen atoms of the alloxazine ring which accept hydrogen. (Other yellow enzymes are known whose prosthetic groups contain adenine also.) Flavoprotein is reduced by the coenzymes and oxidized by the cytochrome c-cytochrome oxidase system.

(4) The *iron porphyrin* enzyme system, cytochrome c-cytochrome oxidase (indophenol oxidase). The precise arrangement of iron atoms and pyrrol rings is not known. It constitutes the outermost link in the respiratory chain, being reduced by flavoprotein and oxidized by oxygen.

(5) *Peroxidase*, which oxidizes substrates with hydrogen peroxide, and *catalase* which breaks down hydrogen peroxide to water and oxygen, appear to be set somewhat apart from the other respiratory enzymes and their function is but poorly understood.

[5] For a detailed discussion see Elvehjem and Wilson: *Respiratory Enzymes.* Burgess Publishing Co., Minneapolis. 1939.

It is to be understood that the "system" represented by these respiratory enzymes is undoubtedly an incomplete picture of the respiratory processes of the living cell, and at the same time to a certain extent a generalization. Coupled oxidation-reduction reactions among metabolites, including intramolecular oxidation and dismutation referred to above, not only occur but are of considerable importance in certain cases such as that of the butyl alcohol-acetone fermentation. Furthermore, any cellular component which may be reversibly oxidized and reduced must be suspected of respiratory function; these include some of the bacterial pigments, glutathione, cystine, and others.

Oxidation-Reduction Potential.[6]—It is evident from the foregoing discussion of anaerobic oxidations and outline of hydrogen transport mechanisms that the terms oxidation and reduction do not necessarily refer to reactions in which oxygen plays a part. In fact, in most biological oxidations the participation of oxygen is, in a sense, incidental to the process.

It continues to be customary in biochemistry to refer to hydrogen transport, *i.e.*, the removal and addition of hydrogen atoms as indicated above. It is more precise, however, to speak of electron transfer. This will be obvious from consideration of the oxidation of an ion, such as the oxidation of iron from the ferrous to the ferric state. Here one electron is removed from the outer orbit and, through removal of one unit negative charge, the charge of the ion is altered from $++$ to $+++$ and the valence changed from two to three, since it is now possible for the ion to share three instead of two electrons with another ion. Since the flow of electrons constitutes an electric current, it is possible to measure reducing intensity or electron-escaping tendency (fugacity) of a system by measuring the electron pressure or electric potential. This may be carried out in practice by immersing an inert metallic electrode in the reaction mixture; electrons set free from ions may enter the electrode, or electrons may leave the electrode to combine with ions in the solution. It will be clear that this is a measure of reducing *intensity*, not of reducing *capacity*.

Such a potential must have some reference point and that adopted for oxidation-reduction potential is the potential of the normal hydrogen electrode. That is to say, the potential of an inert electrode immersed in a solution of *p*H 0 in equilibrium with one atmosphere of hydrogen is taken as zero. The oxidation-reduction potential is designated by the symbol E_h. It may be noted that the oxygen electrode is purely hypothetical.

Let us consider the precise definition of E_h as given by the general electrode equation, without reference to its derivation.[7] The relationship is:

$$E_h = E_o + \frac{RT}{nF} \ln \frac{[ox]}{[red]}$$

E_h and E_o are measured in volts, R is 8.31507 volt coulombs, T is absolute temperature, F is 96,500 coulombs, and n is the number of electrons in-

[6] For a general discussion appropriate here see Hewitt: *Oxidation-Reduction Potentials in Bacteriology and Biochemistry*. 4th ed. London County Council. P. S. King & Son, Ltd. London. 1936.

[7] Clark: Pub. Health Repts., 1923, *38*:443. This and nine additional papers on oxidation-reduction studies are contained in Hygienic Laboratory Bulletin No. 151, 1928.

volved in the oxidation-reduction reaction. In a given system the number of electrons is constant and when that system is maintained at a constant temperature, $\frac{RT}{nF}$ becomes a constant. Thus, if we assume a system in which two electrons are involved at 30° C. and convert to Briggsian logarithms, the expression becomes:

$$E_h = E_o + 0.03 \log \frac{[ox]}{[red]}$$

It is apparent from this that the potential is a function of E_o, a constant for the system, and the ratio of the concentrations of oxidized to reduced form of the compound. When these concentrations are equal, *i.e.*, when the system is half oxidized and half reduced, $E_h = E_o$. Thus the system under consideration may be characterized by the value of E_o and will reduce systems whose E_o is positive to it, and oxidize systems whose E_o is negative to it. The deviation of E_h from the E_o of the system is clearly a function of the relative proportions of oxidized and reduced forms; when the relative concentration of the oxidized form is high, the potential is more positive and *vice versa.*

The Effect of p*H.*—In the above it has been assumed that *p*H remains constant; if it varies the potential is affected. Since either or both of the oxidized and reduced forms of the compound are ionized, alteration in *p*H changes their relative concentrations by altering ionic equilibria. The effect is variable in magnitude, depending upon the number of electrons exchanged and whether ions are created or destroyed. Omitting consideration of detail, it may be pointed out that in, for example, a system in which no ions are created or destroyed, the observed potential becomes increasingly negative with rise in *p*H at a rate of 30 millivolts per unit *p*H when one electron is involved, 60 millivolts when there are two electrons, and so on. Because of this effect, *p*H must be specified for any value of E_h or else the value is meaningless. Similarly, the E_o of a system represents the potential at which the system is half oxidized at *p*H 0; another constant, E'_o, may be derived to represent the potential of a half oxidized system at any specified *p*H.

It would be of considerable advantage, especially in biological systems, to have a measure of reducing intensity independent of changes in *p*H. Such a measure was early developed by the derivation of *r*H. This is analogous to *p*H in that it is the logarithm of the reciprocal of the hypothetical partial pressure of hydrogen gas in equilibrium with the system. In a divalent system at 30° C, for example:

$$E_h = 0.03\ (rH - 2\ pH)$$

Thus, given *p*H and E_h, *r*H may be calculated. This measure has been criticized on many grounds and it is now generally agreed that its use is highly undesirable and should be discontinued.

Application to Biological Systems.—The foregoing treatment of oxidation-reduction potential applies only to completely reversible systems. A number of biological systems are reversible and electromotively active and

therefore susceptible to such analysis. This group includes the hemochromogens and the hemes such as cytochrome and cytochrome oxidase, the flavins, and a number of naturally occurring pigments. A second group is made up of the sluggish systems, so-called because of their sluggish behavior at the electrode, which are regarded as only partially electromotively active. Coenzyme I and the sulfhydryl compounds such as glutathione and cysteine belong to this group, and the measurement of their oxidation-reduction potentials is difficult and generally unsatisfactory. Lastly there are those sluggish systems that require enzymatic catalysis, the enzymatic oxidation-reduction systems. There are a number of these, including succinate-fumarate, lactate-pyruvate, alcohol-acetaldehyde, and others.

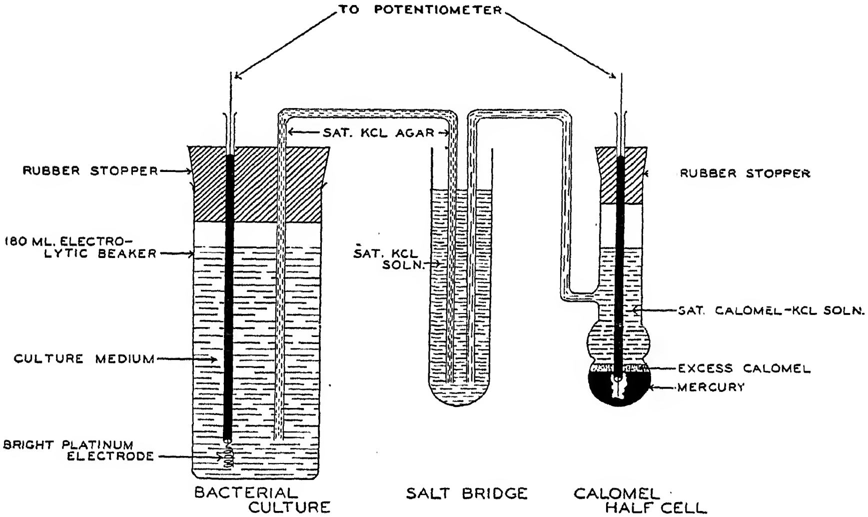


Fig. 14.—Diagrammatic representation of the method used in the measurement of reduction potentials developed in bacterial cultures. The culture vessel should contain duplicate electrodes and an additional opening for inoculation, removal of samples, etc.

Unfortunately, perhaps, many of the oxidation-reduction systems in the living cell fall into this last group, and undoubtedly there are other systems which are not as yet known, as well as still others which are not reversible or only partially so. It becomes very difficult, therefore, to interpret potentials produced by the complex system that is the living cell for, during active metabolism, a number of systems must be functioning simultaneously. As a matter of fact, it is open to question whether the reducing intensities developed should be termed "oxidation-reduction potentials"; Conant[8] has suggested that certain types of potentials measurable by velocity of oxidation, even though the systems be irreversible, be called "apparent oxidation-reduction potentials." Probably reduction potential or

[8] Conant: Chem. Rev., 1926, *3*:1.

reducing intensity are the most satisfactory terms for potentials developed by the actively metabolizing intact cell. It may be noted that those reactions in which oxygen is evolved, usually as peroxide, are somewhat apart from the type of system treated in electrochemical oxidation-reduction theory.

Reduction Potentials of Bacteria.—The development of reducing properties by bacteria is associated with equivalent electrometric and colorimetric changes, indicating that the predominant system or systems are reversible. The potentials may be developed by bacterial suspensions in the presence of added substrate, or during growth in culture; the latter state has been by far the more commonly studied. Measurement of the reducing intensity is made electrometrically, using bright platinum or gold-plated platinum electrodes, as the use of indicator dyes which change color with oxidation or reduction has been unsatisfactory. An arrangement that has been used extensively in the writer's laboratory is illustrated in Fig. 14 in diagrammatic form. It is to be noted that the bacterial culture is a half cell, the standard half cell being a saturated calomel cell here, though a N/10 calomel or a quinhydrone half cell may be used. The whole is immersed in a constant temperature bath. With buffered broth as the culture medium, the *p*H is maintained without significant variation.

The reducing capacity of the bacterial culture is very low, *i.e.*, it is poorly poised (analogous to poorly buffered with respect to changes in *p*H), and if appreciable amounts of current are passed through the system in the measurement of the potential, the electrodes polarize, making readings impossible. It is necessary, therefore, to insert a high resistance in series with the cell; 10 megohms is satisfactory. The small amount of current passed necessitates the use of a highly sensitive null point instrument for its measurement; the ordinary type of potentiometer-electrometer with vacuum tube amplification is adequate. The calculation of the potential is simple:

$$E_h = E_{obs} + E_{std}$$

when E_{obs} is the observed potential and E_{std} the potential of the standard half cell. Measurements with duplicate electrodes should agree within 3 to 5 millivolts. Measurement of the developing potential may be made at intervals to give a time-potential curve.

The potential of freshly prepared sterile culture medium often shows a slight, gradual negative drift. When inoculated with bacteria, however, the potential falls rapidly during the early hours of incubation. Thereafter it differs somewhat with the type of bacterium. In the case of the obligate anaerobes (see below) it reaches and maintains a very low level, sometimes approaching or reaching the level of hydrogen overvoltage, *i.e.*, more negative than the normal hydrogen electrode. Some facultative anaerobes such as the colon bacillus reach similar low levels while others do not. A point of some interest is that the levels attained and maintained by bacteria of the *Salmonella* group are characteristic of species.[9] Other kinds of bacteria behave still differently. Pneumococcus cultures, for example,

[9] Burrows and Jordan: Jour. Inf. Dis., 1935, *56*:255; *ibid.*, 1936, *58*:259.

show a rapid positive drift after a negative potential has been established; this is probably due to the accumulation of peroxide since it does not occur in catalase broth cultures. Representative time-potential curves are given in Fig. 15.

The relation of potential to *p*H becomes of some significance when the *p*H of the culture changes with incubation, as when a fermentable sugar is included in the medium. The effect of changing *p*H cannot be predicted since the functioning oxidation-reduction systems are unknown. This relationship has been determined experimentally for nutrient broth cultures of certain *Salmonella* species, and the observed relation was very close to that of a divalent system with no destruction or creation of ions, *i.e.*, 60 millivolts per unit *p*H.[10]

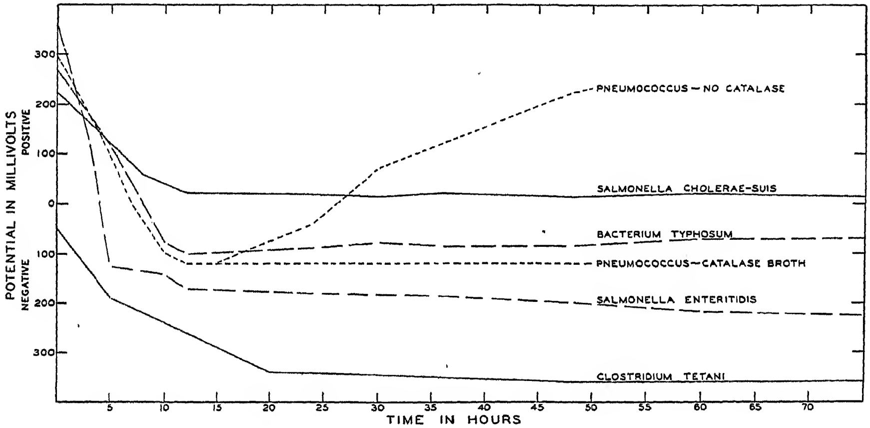


Fig. 15.—Time-potential curves illustrating the development of reducing intensities in bacterial cultures. Note the rise in potential in pneumococcus cultures in non-catalase-containing media resulting from the accumulation of peroxide. The development of species-characteristic potentials is apparent. Pneumococcus data from Hewitt, *Cl. tetani* from Gillespie and Rettger, remainder from Burrows and Jordan.

The Relation of Bacteria to Gaseous Oxygen.—The bacteria differ from one another with respect to their relationship to atmospheric oxygen, and three groups may be distinguished:

(1) *The Obligate Aerobes.*—These bacteria require ready access to air, growing only feebly if at all in its absence. Such microorganisms might be presumed to be deficient in certain of the respiratory enzymes and unable to respire under anaerobic conditions, though there is no proof of this. It is also possible that end-products of their anaerobic metabolism are toxic to them, or that they cannot metabolize in the presence of strong reducing intensities. This group includes such well-known bacteria as the nitrifying bacteria, *Bacillus subtilis* and related forms, the diphtheria bacillus, the cholera vibrio and others.

[10] Burrows: Jour. Inf. Dis., 1942, *71*:106.

(2) *The Facultative Anaerobes.*—Bacteria of this group respire with equal facility in the presence or absence of gaseous oxygen. In broth cultures the dissolved oxygen is soon exhausted and, after a preliminary period of aerobic growth, the culture continues to develop under essentially anaerobic conditions. Such bacteria presumably have a full complement of respiratory enzymes of which they are able to use all or any part.

(3) *The Obligate Anaerobes.*—These bacteria are unable to grow in the presence of atmospheric oxygen and, in fact, oxygen is actively toxic to them; vegetative cells die very quickly upon exposure to air though spores are highly resistant. Microaerophilic forms which tolerate very low concentrations of oxygen are usually included in this group. These bacteria are among the few living organisms which lack the respiratory pigment cytochrome, a fact that is definite evidence of incomplete enzymatic equipment. These forms might be regarded as having carried the process of adaptive differentiation too far and ended in an evolutionary blind alley as have the lungfish. They also show certain peculiarities in respiratory metabolism, bringing about mutual oxidation-reduction of paired amino acids, and many species actively metabolize lactic acid formed in fermentation with the production of compounds such as butyric acid, butyl alcohol and acetone.

The toxicity of atmospheric oxygen to the obligate anaerobes, however, requires further explanation than lack of respiratory enzymes which react with oxygen. Two theories have been proposed:

The Catalase Theory.—This, the earlier theory, is based on the discovery[11] that certain bacteria produce hydrogen peroxide in amounts sufficient to be toxic to themselves. In cultures of those species which do not produce catalase and are peroxide-sensitive, peroxide accumulates and the culture becomes self-sterilizing; the pneumococcus is an example of this type of microorganism. Those bacteria which produce catalase decompose the peroxide as it forms and it does not accumulate to toxic concentrations. The obligate anaerobes are peroxide-sensitive and do not form catalase. Hence it has been suggested that these organisms form peroxide rapidly in the presence of air and therefore oxygen is toxic to them. Although this theory is accepted by some, it entirely lacks supporting evidence, for all attempts to demonstrate peroxide in cultures exposed to air have failed.

The Oxidation-Reduction Potential Theory.—It has also been proposed that the reducing intensity of the medium is the factor governing whether or not growth will occur. Experimental evidence, both direct[12] and indirect,[13] has been presented which indicates that a certain degree of reducing intensity is essential to the germination of tetanus spores; the positive limit appears to be about + 50 millivolts at *p*H 7.0. It has been suggested that this accounts for the failure of tetanus spores to germinate in healthy tissues in the absence of trauma or secondary infection as is sometimes observed. Since a sufficient reducing intensity cannot be maintained in the presence of oxygen, oxygen is toxic. It is not clear why such

[11] McLeod and Govenlock: Lancet, 1921, *i:*900.
[12] Knight and Fildes: Biochem. Jour., 1930, *24:*1496.
[13] Quastel and Stephenson: Biochem. Jour., 1925, *20:*1125.

a reducing intensity should be required. Possibly some of the respiratory enzymes of the obligate anaerobes are oxygen-labile, *i.e.*, active in the reduced state and inactive in the oxidized state, as are some of the bacterial hemolysins (p. 178), or perhaps the substrates of some of the enzymes of these organisms are unstable under aerobic conditions.

THE AUTOTROPHIC BACTERIA

Of the many types of physiological activity occurring among the bacteria, that of the so-called *autotrophes* is unique in biochemical physiology. The respiratory mechanisms of chemosynthetic organisms are concerned, for the most part, with the oxidation of organic molecules. Such organisms are generally spoken of as *heterotrophes*, organisms which are dependent upon the organic substances synthesized by other living cells. The autotrophic bacteria, however, derive the energy necessary to their life processes from the oxidation of inorganic compounds of nitrogen, sulfur and iron, and obtain nitrogen from ammonium salts and carbon from carbonate or carbon dioxide.

The Nitrifying Bacteria.—Of the autotrophic bacteria perhaps the most important single group is that of the microorganisms, discovered by Winogradsky in 1890, which oxidize ammonia to nitrite and nitrite to nitrate. The species of bacteria concerned are *Nitrosomonas* and *Nitrosococcus* (so-called because their cultures give the nitroso reaction in the qualitative test for nitrite) which bring about the reaction:

$$NH_3 \longrightarrow {}^-NO_2$$

and *Nitrobacter* which brings about the reaction:

$${}^-NO_2 \longrightarrow {}^-NO_3$$

These equations represent the entire respiratory mechanism of these organisms; not only do they not oxidize organic material but organic substances are actually toxic to them and in any but minute concentrations completely inhibit growth.[14] It has been shown, however, that they carry on an endogenous metabolism, presumably of intracellular organic matter.[15]

The relative simplicity of the respiratory mechanisms of these two species of nitrifying bacteria has made possible a reliable determination of the free energy efficiency of these organisms. The ratio of nitrogen oxidized to carbon fixed as protoplasm has been found to be 35 for *Nitrosomonas* and 135 for *Nitrobacter*. Assuming the energy consumed in the synthesis of protoplasm to approximate that of the reduction of carbon dioxide to hexose, the efficiency of *Nitrobacter* becomes 7.8 per cent and that of *Nitrosomonas* 5.9 per cent.[16] Presumably, then, about 95 per cent of the energy

[14] For example, 0.015 M glucose completely inhibits *Nitrobacter* in liquid media and somewhat higher concentrations, 0.2 per cent, are necessary to inhibit growth in sand media or soil. (Coleman: Centralbl. f. Bakt., 1908, Abt. II, *20*:401, 485.)

[15] Bömecke: Arch. f. Mikrobiol., 1939, *10*:385.

[16] Calculated on a free energy rather than heat of combustion basis. *Cf.* Bass-Becking and Parks: *Energy Relations in the Metabolism of Autotrophic Bacteria.* Physiol. Rev., 1927, *7*:85.

liberated should appear as heat, and experiment has shown this to be true. As machines, therefore, the nitrifying bacteria are not highly efficient.

The actual process of oxidation is entirely unknown; it has been suggested that hydroxylamine and hyponitrous acid may be intermediates, but there is no evidence for this. There is some evidence that formaldehyde is an intermediate in the reduction of carbonate to protoplasm.[17]

The organisms may be isolated by the inoculation of mineral salt solutions with soil, the nutriment being carbonate and an ammonium salt in the case of *Nitrosomonas* and *Nitrosococcus* and carbonate and nitrite for *Nitrobacter*. Isolation by the usual pour plate pure culture methods is difficult if not impossible, but colonies may be secured by inoculating silica gel plates from the enrichment cultures. *Nitrosomonas* grows in microscopic, colorless colonies which turn brown on continued incubation, while *Nitrobacter* produces somewhat larger colonies, light brown in color.

The oxidation of ammonia is brought about not by a single species of bacterium but rather by a group of closely related organisms. *Nitrosomonas europa*, found in western Europe, is a coccobacillus about 1.5 by 1.0 μ and exists in two forms, one a zooglea of closely packed cells and the other an actively motile swarming (monas) stage in which each cell has a single flagellum. Morphologically similar organisms have been found in this country and elsewhere, although in some cases one stage appears to predominate over the other to such a degree that some might be taken to be different organisms. Coccus forms, given the generic name *Nitrosococcus*, have also been isolated in various parts of the world. A number of species have been reported which some workers group under the single head of *Nitrosococcus nitrosus*. These organisms are all gram-positive, obligate aerobes. The nitrite oxidizing organisms appear to be somewhat more homogeneous. All are rod-shaped, 1.0 by 0.3 to 0.4 μ in size, with one or both ends pointed, non-motile and gram-negative or gram-positive. Neither *Nitrobacter* nor the ammonia oxidizing organisms form spores.

The Sulfur Bacteria.—The group of autotrophic bacteria that derive their energy from the oxidation of sulfur and its compounds is, both morphologically and physiologically, somewhat more complex than that comprising the nitrifying organisms. Some of the organisms, the filamentous forms, are more closely related to the lower fungi than are the so-called "true bacteria." *Beggiatoa*, one of the common forms, shows a close resemblance to the blue-green alga *Oscillaria*. Others, such as *Thiobacillus*, are morphologically indistinguishable from the usual bacteria. In all, three morphological groups may be observed:

(1) The thread-forming, filamentous forms usually regarded as "higher bacteria" which accumulate sulfur granules within the cells. Three genera are ordinarily included in this group: *Beggiatoa*, *Thiothrix* and *Thioplaca*.

(2) Non-thread-forming organisms existing in a number of forms and including such genera as *Thiobacillus*, *Thiospirillum*, *Thiovulum*, *Achromatium*, etc. *Thiobacillus* is often separated from the other organisms on the basis of the failure to accumulate sulfur granules within the cells.

[17] Klein and Svolba: Ztschr. f. Bot., 1926, *19*:65.

(3) The purple and green sulfur bacteria, which differ from the other two groups in that they are pigmented, the pigments making possible a photosynthetic metabolism.

The sulfur bacteria are widely distributed over the earth and are commonly found in the water of sulfur springs, in sewage-laden streams, in swamps where masses of vegetable matter are undergoing slow decomposition and, in fact, wherever sulfur and hydrogen sulfide are present. Whether such organisms bear any relation to the laying down of sulfur deposits is uncertain. It has been suggested that possibly gypsum deposits may have resulted from the microbial oxidation of sulfur to sulfate neutralized by calcium salts present in the earth.

The Sulfide Bacteria.—The oxidation of sulfur compounds by these organisms proceeds through a series of stages. The so-called "sulfide bacteria," including the filamentous forms, derive their energy primarily through the oxidation of hydrogen sulfide to elementary sulfur, which is deposited as granules within the cells:

$$H_2S + \tfrac{1}{2}\,O_2 \longrightarrow H_2O + S$$

The reaction is exothermic (because of the simultaneous oxidation of hydrogen) and represents the respiratory mechanism of these organisms. If the supply of hydrogen sulfide runs low, the organisms further oxidize the accumulated sulfur to sulfate:

$$S + 1\tfrac{1}{2}\,O_2 + H_2O \longrightarrow H_2SO_4$$

The Thionic Acid Bacteria.—A second physiological group, called the thiosulfate or thionic acid bacteria, obtains its energy primarily through the oxidation of thiosulfate, though some members are capable of oxidizing elementary sulfur. The obligate autotrophe, *Thiobacillus thioparus*, is included in this group, together with *Thiobacillus denitrificans*. Some strains of the latter have been found to be obligate autotrophes and also obligate anaerobes, growing in the presence of nitrate, while other strains are both facultative autotrophes and facultative anaerobes. *Thiobacillus thioparus* accumulates free sulfur outside of the bacterial cells when grown in thiosulfate media and the oxidation is supposed to proceed thus:

$$5\,Na_2S_2O_3 + H_2O + 4\,O_2 \longrightarrow 5\,Na_2SO_4 + H_2SO_4 + 4\,S$$

This organism is also able to oxidize sulfide and tetrathionate but oxidizes elementary sulfur to sulfate only very slowly. A number of common enteric bacilli, however, are able to reduce tetrathionate.[18]

The oxidation of sulfur and reduction of nitrate by *Thiobacillus denitrificans* growing under anaerobic conditions proceeds either as:

$$5\,S + 6\,KNO_3 + 2\,H_2O \longrightarrow K_2SO_4 + 4\,KHSO_4 + 3\,N_2$$

or, in the presence of calcium carbonate, as:

$$5\,S + 6\,KNO_3 + 2\,CaCO_3 \longrightarrow 3\,K_2SO_4 + 2\,CaSO_4 + 2\,CO_2 + 3\,N_2$$

[18] Pollock, Knox and Geli: Nature, 1942, *150*:94.

The organism is able to oxidize thiosulfate anaerobically in the presence of nitrate according to the following reaction:

$$5\,Na_2S_2O_3 + 8\,KNO_3 + 2\,NaHCO_3 \longrightarrow 6\,Na_2SO_4 + 4\,K_2SO_4 + 2\,CO_2 + 4\,N_2 + H_2O$$

The Sulfur Bacteria.—A third group, the sulfur bacteria, is represented by only one species, *Thiobacillus thiooxidans*, which derives its energy primarily through the oxidation of elementary sulfur to sulfate. It is also able to oxidize thiosulfate to sulfate, both oxidations proceeding as indicated above. This strictly autotrophic organism is remarkable for its tolerance of high acidities. Sulfuric acid accumulates in its cultures as a result of the oxidation of sulfur, and normal growth takes place in concentrations as high as 0.25 molar, and even 0.5 molar does not completely inhibit growth. Although a strict autotrophe, *Thiobacillus thiooxidans* is not so sensitive to the presence of organic matter as are some other autotrophes and is even able to decompose glucose. The breakdown of glucose, however, does not appreciably alter the sulfur-carbon ratio (the ratio of sulfur oxidized to carbon reduced to protoplasm) and apparently the organism is unable to use the energy so made available. It does, however, metabolize a polysaccharide formed during growth, apparently by a process of phosphorylation.[19] Like the nitrifying bacteria, these organisms also show an endogenous respiration, indicated by slow oxygen uptake, indicating that there is a breakdown of intracellular organic matter.[20]

Although the influence of light on the metabolism of the purple and green sulfur bacteria had been suspected for some time, it is only in recent years that the photosynthetic character of their physiological activities has been demonstrated through the work of van Niel.[21] The organisms grow under strictly anaerobic conditions in the presence of hydrogen sulfide and carbonate. In the absence of oxidizable sulfur compounds purple sulfur bacteria can develop in the presence of organic compounds under completely anaerobic conditions. As in plants, photosynthesis in bacteria is now interpreted as an oxidation-reduction process, and may be expressed by the equations:

$$4(H_2O + \text{light} \longrightarrow H + OH)$$
$$4\,H + CO_2 \longrightarrow (CH_2O) + H_2O$$
$$2(2\,OH + H_2A \longrightarrow H_2O + A)$$

It is to be noted that molecular oxygen is not liberated and the peroxide mechanism of green plants is replaced by one in which hydrogen donators, *e.g.*, hydrogen sulfide, regenerate the system. In the presence of organic matter and the absence of hydrogen sulfide, the reaction is coupled with dehydrogenase systems. It is suggested by van Niel that the photochemical decomposition of water is the light reaction, while the reduction of carbon dioxide is a dark reaction. The green pigment, known as *bacteriochlorin* or *bacteriochlorophyl*, is a pyrrol pigment with a porphin nucleus containing

[19] LePage: Arch. Biochem., 1942, *1*:255; also LePage and Umbreit: Jour. Biol. Chem., 1943, *148*:255.
[20] Vogler: Jour. Gen. Physiol., 1942, *25*:617.
[21] *Cf.* the review by van Niel: Advances in Enzymology, 1941, *1*:263.

magnesium, and it is closely related to, though not identical with, green plant chlorophyls *a* and *b*.

A group of organisms known as the non-sulfur purple bacteria contain bacteriochlorophyl and are photosynthetic, but differ from the purple sulfur bacteria in that the reduction is coupled only with the oxidation of organic matter, *i.e.*, they are not facultative autotrophes. They are, therefore, intermediate between the sulfur forms and non-photosynthetic bacteria with a typical oxidative metabolism. Available information concerning them has been reviewed by van Niel.[21a]

The bacterial oxidation of sulfur and its compounds is of considerable importance in the processes involved in the maintenance of the sulfur cycle. As in the case of nitrogen, many bacteria are capable of reducing sulfur—the evolution of hydrogen sulfide from decomposing organic matter reflects the widespread reduction of sulfur in lieu of oxygen under anaerobic conditions—but only a few are able to oxidize it. These organisms assume considerable practical significance at times, producing corrosion of metals laid in sulfur-containing clay soils[22] and are sometimes responsible for the disintegration of stone and mortar.[23]

The relative simplicity of the respiratory mechanisms of the sulfur bacteria is in itself of no small general physiological interest, but equal importance attaches to the fact that these organisms appear to constitute a link between the autotrophic organisms and those dependent upon preformed organic matter through the species that are facultatively autotrophic. Their efficiency is of the same order as that of the nitrifying bacteria, *i.e.*, 5 to 8 per cent of the energy made available through the oxidation of sulfur and sulfur compounds is utilized in synthesis of cell substances.

The Iron Bacteria.—Certain of the filamentous bacteria are characterized by deposits of ferric hydroxide in the sheath surrounding the filament or, sometimes, within the cell itself. It was early suggested that these organisms are able to oxidize ferrous to ferric iron and utilize the energy so liberated. It appears, however, that although some of the so-called iron bacteria are obligate autotrophes which are dependent upon the oxidation of iron as a source of energy, the presence of ferric hydroxide is not necessarily indicative of an autotrophic metabolism. One species, *Spirophyllum ferrugineum*, is an obligate autotrophic organism which grows in inorganic solutions containing ferrous carbonate. The oxidation proceeds according to the following reaction:

$$2\,FeCO_3 + 3\,H_2O + \tfrac{1}{2}\,O_2 \longrightarrow Fe_2(OH)_6 + 2\,CO_2$$

The reaction is exothermic but the energy yield is small, about 15 calories per mol, and large amounts of carbonate must be oxidized. Some of the iron bacteria are also capable of oxidizing manganese salts and precipitate manganese hydrates in their cells.

Other iron bacteria, however, can live without iron and are able to util-

[21a] van Niel: Bact. Rev., 1944, *8*:1.

[22] *Cf.* Bunker: Jour. Soc. Chem. Ind., 1939, *58*:93.

[23] Gistl: Centralbl. f. Bakt., Abt. II, Orig., 1940, *102*:486.

ize either ferrous carbonate or soluble iron salts of organic compounds. Still others use organic iron compounds but not inorganic iron salts. Harder[24] divides the iron bacteria into three physiological groups:

(1) those, such as *Spirophyllum ferrugineum*, that precipitate ferric hydroxide from solutions of ferrous carbonate and use the carbon dioxide liberated and the energy produced during the oxidation for their life processes (obligate autotrophes);

(2) those, such as *Leptothrix ochracea*, that do not require ferrous carbonate but that cause the deposition of ferric hydroxide when either inorganic or organic iron salts are present (facultative autotrophes); and

(3) those, probably including a number of the lower or ordinary water and soil bacteria, that attack organic iron salts, using the organic acid radical as food and precipitating ferric hydroxide or basic ferric salts, which are gradually changed to ferric hydroxide (heterotrophes).

The best known of the iron bacteria are the *Spirophyllum* and *Leptothrix* species indicated above and *Crenothrix polyspora*. These organisms sometimes grow in the conduits of certain public water supplies, where they form unpleasant-looking, brownish, flocculent masses, often leading to complete stoppage of the pipes. The frequent appearance of detached portions of the growth in tap water may give rise to consternation among the water consumers, as in the famous "water calamities" in Berlin, Lille, Rotterdam and other places. There is no evidence that such organisms are directly harmful.

There seems little doubt that iron-depositing bacteria have played an important part in the formation of iron ore deposits, but the relative share of chemical and biological processes in an individual case can be determined only by a thorough study of conditions at the time of deposition, especially as regards sedimentation, climate, depth of water, nature of material in solution and other factors.[24]

The Oxidation of Hydrogen.—The bacterial oxidation of hydrogen has already been indicated in the case of the oxidation of hydrogen sulfide to elementary sulfur. Other organisms, given the generic name *Hydrogenomonas*, are, however, obligate autotrophes which depend upon the oxidation of hydrogen as a source of energy for synthesis. The first of these organisms was described in 1906 by Kaserer[25] and named *Hydrogenomonas pantotropha*. Other species were described later, such as *Hydrogenomonas vitrea* and *Hydrogenomonas flava*. The oxidation of hydrogen:

$$H_2 + \tfrac{1}{2}\, O_2 \longrightarrow H_2O$$

yields relatively large amounts of energy (34.2 Cal. per gram as contrasted with 4.1 Cal. per gram of starch) and, although it cannot be accurately measured, the free energy efficiency of these organisms appears to be somewhat higher than that of the other autotrophes, *i.e.*, 10 to 20 per cent.

[24] Harder: U. S. Geological Survey, Professional Paper 113, Washington, 1919. See the review by Dorff: Pflanzenforschung, 1934, Heft 16.

[25] Kaserer: Centralbl. f. Bakt., 1906, Abt. II, *16*:681.

It has been suggested that all the hydrogen is not burnt directly by these organisms but that a part of it is converted to formaldehyde, which may in turn be either oxidized or used for synthesis:

$$H_2CO_3 + 2\ H_2 \longrightarrow HCHO + 2\ H_2O$$
$$HCHO + O_2 \longrightarrow H_2CO_3$$

Although most of the hydrogen bacteria are obligate aerobes, some are able to grow anaerobically in the presence of nitrate. Other studies[26] have shown that other species are present in the soil which are facultative autotrophes, being able to live on organic matter in the absence of hydrogen.

As in the case of the oxidation of iron, the oxidation of hydrogen is not confined to a few species of strict autotrophes. Recent work[27] has shown that a number of well-known heterotrophic bacteria such as *Bacterium coli*, *Bacterium prodigiosum*, *Bacillus megatherium*, *Bacillus subtilis*, *Clostridium sporogenes*, *Azotobacter* and others are able to use gaseous hydrogen. The enzyme activating molecular hydrogen is named hydrogenase. In one case, an organism of the colon-typhoid group, the oxidation of hydrogen is accompanied by a reduction of sulfate to sulfide, and possibly this reaction may be a general one.

The Oxidation of Carbon Monoxide.—The bacterial oxidation of carbon monoxide, comparable in many respects to the oxidation of hydrogen, was reported by Beijerinck and van Delden.[28] These workers isolated an organism which they named *Bacillus oligocarbophilus*, an obligate autotrophe which grew in the absence of organic carbon compounds and obtained its nitrogen from ammonia, nitrite or nitrate. It was later shown[29] that this organism derived its energy through the oxidation of carbon monoxide to carbon dioxide:[30]

$$CO + \tfrac{1}{2}O_2 \longrightarrow CO_2$$

HETEROTROPHIC BACTERIA

It is seldom if ever possible to draw sharp lines of demarcation in biology, and the separation of bacteria into autotrophic and heterotrophic forms being no exception, it must necessarily be to some degree dogmatic. It has already been pointed out that some of the sulfur, iron and hydrogen bacteria are facultative autotrophes, that they may live through the oxidation of either inorganic or organic compounds. Such forms may well be regarded as connecting links between the two physiological types. A somewhat different kind of interconnection is the sharing, between obligate autotrophes and obligate heterotrophes, of a common oxidative

[26] Grohman: Centralbl. f. Bakt., 1924, Abt. II, *61*:256; Ruhland: Jahrb. wiss. Bot., 1924, *63*:321.

[27] Stephenson and Stickland: Biochem. Jour., 1931, *25*:205, 215.

[28] Beijerinck and van Delden: Centralbl. f. Bakt., 1903, Abt. II, *2*:33.

[29] Kaserer: Centralbl. f. Bakt., 1906, Abt. II, *16*:681.

[30] Distilled water standing in the laboratory for long periods may acquire immunologic properties presumably owing to the growth of bacteria. Organisms able to develop on ammonia and carbon monoxide absorbed from the laboratory air may, in some cases, account for such growth on distilled water, a medium which might be supposed to be entirely lacking in nutriment.

mechanism such as the ability to utilize gaseous hydrogen and, under certain conditions, the assimilation of carbon dioxide. Clearly, then, the distinction between the two physiological types is not a sharp one even though the concepts autotrophe and heterotrophe remain extremely useful.

The Oxidation of Simple Organic Compounds. A number of bacteria are able to grow in solutions of inorganic salts, including an ammonium salt, which contain but a single organic compound of one or two carbon atoms. The isolation of an organism, *Methanomonas methanicus*, which would oxidize methane to carbon dioxide and water was reported by Söhngen[31] in 1906.[32] Certain other common bacteria such as *Pseudomonas pyocyanea* and *Pseudomonas fluorescens*[33] are also able to oxidize methane; and one, *Bacillus hexacarbovorum*, is able to utilize not only methane but toluene, xylol and illuminating gas as the sole sources of carbon.[34] Other aliphatic and aromatic hydrocarbons present in petroleum, paraffin oil and similar mixtures are readily used by a number of soil bacteria as sources of energy.[35] These organisms have been isolated on media containing *n*-hexane, naphthenes such as cyclohexane and 1-3 dimethylcyclohexane, or paraffin oil as the only source of carbon. Other organisms capable of oxidizing phenol to carbon dioxide, pyrocatechin to oxyquinon, benzol to fatty acids and carbon dioxide, and the like have been isolated from the soil. Organisms oxidizing the higher aliphatic compounds have been referred to as *paraffinbakterien* by some of the German workers.

Returning to the oxidation of the simpler compounds, another organism, *Bacillus methylicus*, could be grown on the usual laboratory media and, in addition, in solutions of inorganic salts containing formic acid, formaldehyde or methyl alcohol. Organisms such as this are often placed with the autotrophic bacteria, possibly because it is not generally understood that the ability to grow on solutions of inorganic salts plus a single organic carbon compound is one that is possessed by a variety of heterotrophic bacteria. One of the acid-fast bacteria has been grown on methyl alcohol, and a number of bacteria, including the so-called acetic acid or vinegar bacteria, will grow on ethyl alcohol as a sole source of carbon and energy. A number of the paratyphoid bacilli will grow in the absence of all organic compounds other than acetate[36] and certain strains of Friedländer's bacillus will grow in solutions of inorganic salts and formate.[37] Since the ability to grow in simple solutions containing inorganic salts (including an ammonium salt) and a single organic carbon compound is one that is common

[31] Söhngen: Centralbl. f. Bakt., 1906, Abt. II, *15*:513.

[32] For more recent studies see Barker: Jour. Biol. Chem., 1941, *137*:153.

[33] *Cf.* Söhngen: Bot. Centralbl., 1907, *105*:371, and Aiyer: Mem. Dept. Agr. India, Chem. Ser., 1920, *5*:177. See also Munz: *Zur Physiologie der Methanbakterien*, Diss. Halle, 1915.

[34] Störmer: Jahresb. Ver. angew. Bot., 1907, *5*:113.

[35] Bushnell and Haas: Jour. Bact., 1941, *41*:653. Johnson, Goodale and Turkevich: Jour. Cell. & Comp. Physiol., 1942, *19*:163; Grant and ZoBell: Proc. Soc. Exp. Biol. Med., 1942, *51*:266; Stone, Fenske and White: Jour. Bact., 1942, *44*:169.

[36] Braun and Cahn-Bronner: Biochem. Ztschr., 1922, *131*:226 and 272. See also den Dooren de Jong: Diss. Rotterdam, 1926.

[37] Burrows: unpublished experiments.

to a great variety of bacteria usually regarded as heterotrophes, it can hardly be regarded as indicative of an autotrophic metabolism.

The Hydrolysis of Polysaccharides.—Perhaps the richest source of energy available to bacteria is the carbohydrate synthesized, for the most part, by plants and present in nature in the form of complex polysaccharides such as cellulose, hemicelluloses, pectins, starches and the like. Although it has been suggested that the direct oxidation of these compounds may occur by bacterial agency, the preponderance of evidence indicates that hydrolysis of these substances to their component monosaccharides is an essential preliminary to oxidation. The bacterial fermentation of di- and polysaccharides such as sucrose, lactose, starch and inulin consists, then, not only of the actual decomposition to carbon dioxide, organic acids and the like but also includes a preliminary hydrolysis.[38] The hydrolyses are exothermic reactions, but the yield of energy is extremely small as compared to that made available through the oxidation of the component monosaccharides.

Cellulose.[39]—Although cellulose is the most widely distributed polysaccharide in nature, the ability to bring about its decomposition is possessed by only a few organisms. The enzyme cellulase is found in a few higher organisms, some snails and a few marine forms, but even the herbivorous animals are dependent upon the presence of cellulose-decomposing microorganisms in the intestine for the preliminary decomposition of this polysaccharide.[39a] The breakdown of cellulose and the other polysaccharides in nature is an essential part of the decomposition of plant residues and the consequent returning to the atmosphere of carbon dioxide removed through the photosynthetic activities of chlorophyl-containing plants. Relatively few species of bacteria are able to decompose cellulose, but those that do are widely distributed in the soil and the mud of sea bottoms as well as universally in the intestines of herbivorous animals.

A number of bacteria, both aerobic and anaerobic forms, have been isolated which decompose cellulose actively in pure culture. Perhaps the best known aerobic organism is a spiral form, *Spirocheta cytophaga,* which occurs in soil in considerable numbers. A large number of other bacteria which bring about the aerobic decomposition of cellulose have been studied, but the identity of many of them is doubtful. The breakdown of cellulose by anaerobic bacteria is ordinarily accompanied by further decomposition of the glucose so formed to hydrogen and methane in some cases and to organic acids and carbon dioxide in others. Some species have been described, such as *Bacillus cellulosae dissolvens*, which was isolated from human feces, but in general these organisms are not well known. Equally obscure are the thermophiles which have been isolated from actively fermenting manure heaps. The decomposition of cellulose in nature is largely, if not entirely, a process brought about by mixed cultures of bacteria.

[38] See Kluyver and Custers: Antonie van Leeuwenhoek Jour. Microbiol. & Serol., 1940, *6:*121.

[39] See the reviews by Waksman: Bot. Rev., 1940, *6:*637; Norman and Fuller: Advances in Enzymology, 1942, *2:*239.

[39a] *Cf.* Hastings: Bact. Rev., 1944, *8:*235.

The hydrolysis of cellulose takes place in two stages: first, a breakdown to the disaccharide cellobiose; and, second, the hydrolysis of cellobiose to glucose. These reactions are brought about by two separate enzymes, cellulase and cellobiase respectively, and the decomposition, to cellobiose at least, takes place outside the bacterial cell.

Hemicelluloses and Pectins.—In addition to cellulose proper, plant tissue contains other polysaccharide material designated as hemicellulose and pectin. Both are hydrolyzed to sugars, including both pentoses and hexoses, and uronic acids. Although hemicelluloses are decomposed in nature, largely under the influence of bacteria present in the soil, the chemistry of these compounds is largely unknown and, in consequence, little is known of the processes of their decomposition.[40]

The decomposition of pectin, through the agency of the enzyme pectinase, is brought about by a comparatively small number of species of anaerobic bacteria. The process itself is of considerable commercial importance since these organisms are responsible for the retting of flax and hemp and for the rotting of certain fruits and vegetables. Retting is accomplished by submerging flax and hemp in water and allowing the plant tissue to remain until the dissolution of pectin allows the separation of the fibers. The organisms responsible for the decomposition of pectin are closely related to the group of anaerobic soil forms, the amylobacter group, which includes the butyric acid organisms and others. A number of other bacteria, including *Lactobacillus*, *Aerobacillus*, *Micrococcus* and *Enterococcus*, decompose pectins.[41] The nature of the process of decomposition is obscure, though the sugars resulting from hydrolysis are further decomposed to the usual products of sugar oxidation. Few if any of the pectin-decomposing bacteria are able to hydrolyze cellulose also. The combination of the two properties in a single organism would, of course, result in the decomposition of the flax fibers freed by hydrolysis of the pectin.

Starch.—Unlike cellulose, starch may be broken down by a wide variety of living organisms, and the extracellular enzyme diastase, or amylase, is possessed by many bacteria. Like cellulose, however, starch is hydrolyzed in two stages, first to the disaccharide maltose and, second, by a further hydrolysis to glucose. The ability of a given organism to hydrolyze starch has acquired an importance equal to sugar fermentations as a differential character in the study of some bacteria.

Miscellaneous Polysaccharides.—A wide variety of gums and mucilaginous substances, polymers designated as pentosans, levulans, galactans and the like according to the nature of the products of their hydrolysis, may be decomposed by bacteria. Polysaccharides of bacterial origin, such as pneumococcus polysaccharide, may be decomposed by other bacteria (p. 349), and bacteria have been found[42] which hydrolyze agar, a polysaccharide resistant to the action of the great majority of bacteria.

[40] Waksman: *Humus*. Williams & Wilkins Company. Baltimore. 1936.

[41] Werch *et al.*: Jour. Inf. Dis., 1942, *70*:231.

[42] *Cf.* Gray and Chalmers: Ann. Appl. Bot., 1924, *11*:324; Lundestad: Centralbl. f. Bakt., 1928, Abt. II, *75*:321; Nichols: Centralbl. f. Bakt., 1933, Abt. II, *88*:177. Stanier: Jour. Bact., 1944, *42*: 572.

The Oxidation of Carbohydrates.—The ability of bacteria to ferment various carbohydrates to organic acids and, sometimes, carbon dioxide and hydrogen, has proved to be a valuable means of differentiating these organisms from one another. In ordinary description it is customary to state that the typhoid bacillus, for example, does not ferment lactose, that *Staphylococcus aureus* ferments lactose with the production of acid, and that *Bacterium coli* ferments lactose with the production of acid and gas.

The term "fermentation" has been used, and to some extent is still used, to express various conceptions. The tendency at present is to limit it to the disintegration of carbohydrate substances, and there are some who would go so far as to consider as true fermentations only those carbohydrate decompositions in which gas is produced, a virtual reversion to the old etymological signification (*fervere*, to boil). Generally, however, a carbohydrate oxidized to acids, with or without the evolution of gas, is said to be fermented. It may be noted that proteolytic action is not usually denoted as fermentation, though logically the putrefaction of protein substances by bacterial agency falls in the same category with the decomposition of sugar by these microorganisms.

Many organisms are unable to oxidize monosaccharides other than hexoses, but a variety of sugars, including pentoses and a series of higher polyhydric alcohols, can be fermented by bacteria. Most microorganisms display a high degree of specificity with regard to the optical activity of fermentable sugars. Ordinarily the naturally occurring form is actively fermented, while the isomer is fermented only slowly if at all; but this does not always hold true. The resolution of racemic mixtures of oxidizable compounds is not infrequently accomplished by differential fermentation; Pasteur, it will be recalled, separated the *dextro* and *levo* forms of tartaric acid by the fermentation and consequent destruction of one form by a mold.

The end products of the bacterial oxidation of carbohydrates include the non-volatile acid lactic acid, various volatile acids such as formic, acetic, propionic and butyric acids, ethyl alcohol, acetone, butyl alcohol, glycerol, acetylmethylcarbinol, 2,3-butylene glycol, carbon dioxide and hydrogen. The kind and quantity of oxidation products that accumulate in a fermenting mixture depend upon the species of bacterium bringing about the fermentation and the conditions under which the decomposition occurs, such as acidity and the like. Most of the disease-producing bacteria decompose carbohydrate to lactic, formic and acetic acids together with a small amount of ethyl alcohol, and with or without the evolution of gas which may be hydrogen or carbon dioxide or a mixture of both. The higher volatile acids, acetone, glycol and similar compounds are usually associated with the fermentations brought about by soil bacteria. Glucose may be oxidized by bacteria which do not ferment it, and some have distinguished the process of oxidation from that of fermentation.[43]

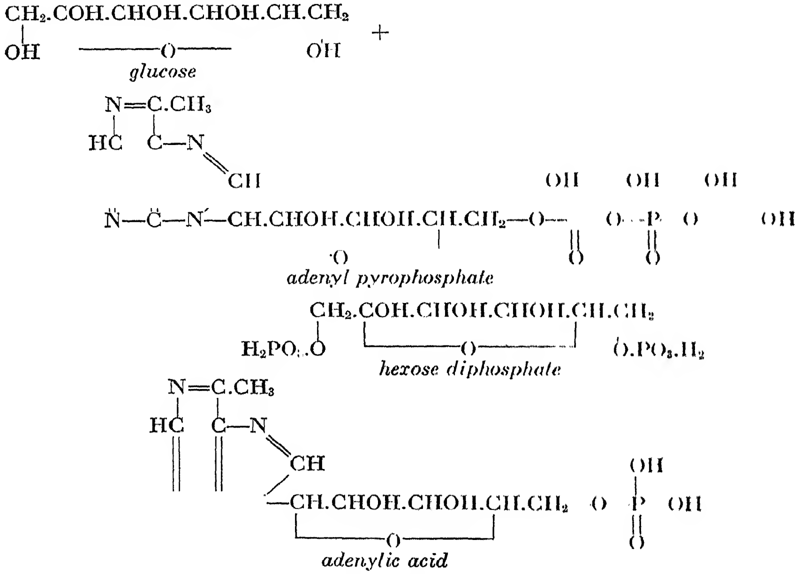
The obvious questions that arise in connection with fermentations are concerned with the way in which decomposition may occur to give

[43] Barron and Friedemann: Jour. Biol. Chem., 1941, *137*:593.

rise to the observed end products. Knowledge of the mechanisms operative in the bacterial decomposition of carbohydrates is extremely fragmentary at the present time. The alcoholic fermentation of glucose by yeast is, however, relatively well understood. Available evidence indicates that the microbial decomposition of sugars is probably of the same general order and, in the present instance is, perhaps, best approached through a consideration of the mechanisms of the alcoholic fermentation followed by a discussion of the probable means by which the observed products of bacterial fermentation arise.

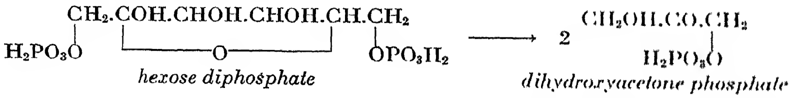
The alcoholic fermentation proceeds in the following manner:

(1) The first step in the decomposition is the phosphorylation of glucose by adenyl pyrophosphate:



The function of the adenyl pyrophosphate is of particular interest in connection with the relatively large amounts of nucleic acid found in the bacterial cell and in the quantitative predominance of adenine over the other bases present.

(2) The hexose diphosphate is split into two three-carbon intermediates or so-called triose phosphates:



(3) The dihydroxyacetone phosphate so formed then reacts with acetaldehyde, the former being oxidized to 3-phosphoglyceric acid and the latter reduced to ethyl alcohol:

$$\begin{array}{ccccccc} \begin{array}{c} CH_2OH.CO.CH_3 \\ | \\ H_2PO_3O \end{array} & + & CH_3CHO & \longrightarrow & \begin{array}{c} COOH.CHOH.CH_2 \\ | \\ H_2PO_3O \end{array} & + & CH_3CH_2OH \\ \textit{dihydroxyacetone phosphate} & & \textit{acetaldehyde} & & \textit{3-phosphoglyceric acid} & & \textit{ethyl alcohol} \end{array}$$

(4) The 3-phosphoglyceric acid is converted to 2-phosphoglyceric acid:

$$\begin{array}{ccc} \begin{array}{c} COOH.CHOH.CH_2 \\ | \\ H_2PO_3O \end{array} & \longrightarrow & \begin{array}{c} COOH.CH.CH_2OH \\ | \\ OPO_3H_2 \end{array} \\ \textit{3-phosphoglyceric acid} & & \textit{2-phosphoglyceric acid} \end{array}$$

(5) Which is then converted to phosphopyruvic acid:

$$\begin{array}{ccc} \begin{array}{c} COOH.CH.CH_2OH \\ | \\ OPO_3H_2 \end{array} & \xrightarrow{-HOH} & \begin{array}{c} COOH.C{=}CH_2 \\ | \\ OPO_3H_2 \end{array} \\ \textit{2-phosphoglyceric acid} & & \textit{phosphopyruvic acid} \end{array}$$

(6) The latter gives up its phosphate to adenylic acid, thereby regenerating adenyl pyrophosphate, and liberating pyruvic acid enol which exists in equilibrium with pyruvic acid:

$$\begin{array}{c} CH_2{=}C.OOH \\ | \\ OPO_3H_2 \end{array} + \text{adenylic acid} \longrightarrow \text{adenyl pyrophosphate}$$
$$+ \; CH_2{=}COH.COOH \rightleftarrows CH_3CO.COOH$$

(7) Pyruvic acid is then decomposed to carbon dioxide and acetaldehyde, the latter becoming available for reaction with dihydroxyacetone as in (3):

$$CH_3COCOOH \longrightarrow CH_3CHO + CO_2$$

Clearly, then, the alcoholic fermentation is a cyclic process in which glucose is consumed and alcohol and carbon dioxide given off. The actual process is very likely somewhat more complex than indicated here; some of the reactions, for example, probably involve as yet unknown intermediates. As it stands, however, the proposed mechanism rests upon a firm experimental foundation; most of the intermediate compounds indicated have been isolated and some have been made available synthetically.[44]

The mechanism developed for the alcoholic fermentation fails to account for the presence of many of the end products of bacterial fermentations. It seems probable, however, that many bacterial fermentations involve a preliminary phosphorylation followed by breakdown into three-carbon compounds which are, in turn, precursors of the organic acids and other compounds formed. It should be noted that other schemes have been developed, particularly by Neuberg,[45] and it is likely that the means by which some bacteria oxidize carbohydrates differ from the above mechanism in a number of respects. It is not entirely certain, for example, that phosphorylation is an inevitable preliminary to further breakdown; in the

[44] For details see the reviews by Nord: Chem. Rev., 1940, *26*:423; and by Meyerhof: Wallerstein Lab. Communic., 1942, *5*:181.

[45] *Cf.* Harden: *Alcoholic Fermentation.* Longmans, Green and Co. London. 1932. Also Schoen: *Le problème des fermentations.* Masson et Cie. Paris. 1926.

fermentation of three and four carbon compounds such as glycerol, fumaric and tartaric acids, phosphate is apparently not involved. The position of methyl glyoxal in the fermentation of hexoses is anomalous, and the widely occurring enzyme glyoxalase[46] (which converts aldehydes into the corresponding hydroxy acids) does not fit into many of the current concepts of the mechanism of sugar oxidation. In spite of an imposing array of evidence, the fundamental questions regarding the mechanisms of the biological oxidation of carbohydrate are answered at best in a piecemeal fashion. It will not be surprising, therefore, that precise information concerning the bacterial fermentations is, for the most part, lacking and postulated mechanisms are often hardly more than hypotheses.

The hexose molecule, whether present as such or resulting from the hydrolysis of a polysaccharide, is split, probably as a phosphoric acid ester, into two three-carbon compounds. Although one of the triose phosphates may break down directly to ethyl alcohol as in the yeast fermentation, a three-carbon compound, currently supposed to be pyruvic acid but in the older literature methyl glyoxal, is formed which serves as a precursor of the end products formed by various species of bacteria.

Lactic Acid.—The lactic acid fermentation is a common one. Probably the great majority of bacteria form some lactic acid, while some, such as *Streptococcus lactis*, the lactobacilli and some of the pathogenic coccus forms as the hemolytic streptococci and the gonococcus and pneumococcus, produce large amounts of this acid from glucose. Although theoretically one of the simplest fermentations, it is one of the least understood. The two more obvious possibilities are each supported in some degree by experimental evidence. One, the conversion of methyl glyoxal to lactic acid under the influence of glyoxalase

$$CH_3.CO.CHO \xrightarrow{HOH} CH_3.CHOH.COOH$$

by the addition of water or through a rearrangement of the hydrated form, is not unlikely. A second possibility is the reduction of pyruvic acid

$$CH_3.CO.COOH \xrightarrow{2\ H} CH_3.CHOH.COOH$$

There is some evidence that this reduction may be coupled with the oxidation of triose phosphate to 3-phosphoglyceric acid in muscle tissue, but there is no evidence of this particular coupling in bacterial fermentations.

Formic Acid.—Formic acid may result from the dismutation of pyruvic acid with the simultaneous production of acetic acid

$$CH_3.CO.COOH \xrightarrow{HOH} CH_3COOH + HCOOH$$

or from methyl glyoxal with the concurrent formation of acetaldehyde as postulated in some of the earlier schemes

$$CH_3.CO.CHO \xrightarrow{HOH} CH_3.CHO + HCOOH$$

[46] *Cf.* Dakin: *Oxidation and Reduction in the Animal Body.* Longmans, Green and Co London. 1922.

or it may arise as a result of the oxidation of acetic acid

$$CH_3.COOH \xrightarrow{2\,O} 2\ HCOOH$$

There is no conclusive evidence, however, as to the immediate precursors of formic acid, nor with regard to the mechanisms of its formation. More recently it has been shown that carbon dioxide may be reduced with gaseous hydrogen by some of those bacteria which activate hydrogen, or by some hydrogen carrier in the case of bacteria lacking hydrogenase, *viz.:*

$$CO_2 + H_2 \longrightarrow HCOOH$$
$$CO_2 + 2\ HA \longrightarrow HCOOH + 2\ A$$

Possibly all of the mechanisms indicated may be operative, perhaps more than one simultaneously in a given fermentation.

Acetic Acid.—Acetic acid may be formed by the dismutation of acetaldehyde

$$CH_3.CHO \xrightarrow{HOH} CH_3.COOH + CH_3.CH_2.OH$$

with the concurrent formation of ethyl alcohol, a reaction which is known to be brought about by a number of bacteria; or it may be formed from pyruvic acid together with formic acid as noted above. The direct oxidation of pyruvic acid to acetic acid and carbon dioxide

$$CH_3.CO.COOH \xrightarrow{O} CH_3.COOH + CO_2$$

has also been demonstrated. The formation of acetic acid is, of course, the basis of the so-called vinegar fermentation and is of considerable practical importance.[47]

Propionic Acid.—Some bacteria, the so-called propionic acid organisms, ferment hexoses to predominantly propionic and acetic acids. The intermediate compound here appears to be lactic acid which is reduced to propionic acid, and acetic acid is formed via pyruvic acid and acetaldehyde

$$CH_3.CHOH.COOH \xrightarrow{-2\,H} CH_3.CO.COOH$$
$$CH_3.CO.COOH \longrightarrow CH_3.CHO + CO_2$$
$$CH_3CHO \xrightarrow{O} CH_3COOH$$
$$CH_3.CHOH.COOH \xrightarrow{2\,H} CH_3.CH_2.COOH + H_2O$$

Butyric Acid.—The fermentation of six-carbon sugars to four-carbon compounds is of no little interest in that the question arises as to whether the six-carbon chain is split into two- and four-carbon intermediates or whether a condensation occurs which will give rise to a four-carbon end product. It seems established that a condensation does take place, probably between two molecules of acetaldehyde

$$2\ CH_3CHO \longrightarrow CH_3.CHOH.CH_2.CHO \longrightarrow CH_3.CH{=}CH.CH(OH)_2 \longrightarrow CH_3.CH_2.CH_2.COOH$$

[47] For a general consideration see Vaughn: Wallerstein Lab. Communic 1942, 5:5.

and in the case of this particular fermentation no unequal splitting of the hexose carbon chain occurs.

Butyl Alcohol and Acetone.—Butyric acid is usually accompanied by butyl alcohol, from which it arises by reduction

$$CH_3.CH_2.CH_2.COOH \xrightarrow{4\ H} CH_3.CH_2.CH_2.CH_2OH + H_2O$$

The production of four-carbon compounds such as butyl alcohol and butyric acid is often associated with the formation of acetone. The origin of acetone in these fermentations is of particular interest and of no small commercial importance. In some cases acetone may be formed through the condensation of two molecules of acetic acid followed by decarboxylation

$$2\ CH_3.COOH \longrightarrow CH_3.C(OH)_2.CH_2.COOH \longrightarrow$$
$$CH_3.CO.CH_2.COOH \longrightarrow CH_3.CO.CH_3 + CO_2$$

or, in other cases, acetaldehyde appears to be the precursor, acetone arising through an aldol condensation

$$2\ CH_3.CHO \longrightarrow CH_3.CHOH.CH_2.CHO \xrightarrow{O} CH_2.CHOH.CH_2.COOH \xrightarrow{2\ H}$$
$$CH_3.CO.CH_2.COOH \longrightarrow CH_3.CO.CH_3 + CO_2$$

The series of oxidations is balanced by simultaneous reduction of acetaldehyde to ethyl alcohol.

Succinic Acid.—The fermentative formation of succinic acid, like the butyric fermentation, involves the production of a four-carbon compound. The mechanisms of this fermentation are obscure. It has been suggested, on the basis of a certain amount of experimental evidence, that succinic acid arises through a cleavage of the hexose molecule into two- and four-carbon compounds which are the precursors of succinic and acetic acids and ethyl alcohol. This is not possible, however, when succinic acid is formed by the fermentation of glycerol, as by the propionic acid bacteria. Here it has been established (see p. 125) that succinic acid is formed by a condensation of carbon dioxide with a three-carbon intermediate, possibly pyruvic acid or a derivative such as the phosphate. The demonstration of the heterotrophic assimilation of carbon dioxide is of primary importance, and since it was first established by Wood and Werkman in the formation of succinic acid by bacteria, it has been found to occur even among higher organisms.

Ethyl Alcohol.—Experimental evidence indicates that alcohol may be formed in a variety of ways. The direct origin from a triose phosphate in the yeast fermentation has already been pointed out. In bacterial fermentations, however, the immediate precursor of alcohol is probably most often acetaldehyde. The reduction of acetaldehyde to alcohol in the acetone fermentation probably occurs, and it is often formed through a dismutation of acetaldehyde to alcohol and acetic acid.

Acetylmethylcarbinol (Acetoin) and 2,3-Butylene Glycol.—The formation of acetylmethylcarbinol by *Bacterium aerogenes* constitutes an important differential character which separates these organisms from *Bacterium coli*

(p. 375). It is probable that this compound is formed through a direct condensation of two molecules of acetaldehyde

$$2\ CH_3.CHO \longrightarrow CH_3.CHOH.CO.CH_3$$

under the influence of an enzyme possessed by *aerogenes* but not by *coli*. Under reducing conditions the compound is reduced to 2,3-butylene glycol or, rarely, it may be oxidized to diacetyl.

Hydrogen.—The gas of the "acid and gas" bacterial fermentations consists of hydrogen and carbon dioxide, the proportions of which are sometimes of differential significance (p. 375). Carbon dioxide may arise in a number of ways during the course of fermentation as already indicated. The production of hydrogen is, however, generally associated with the formation of formic acid, which decomposes to carbon dioxide and hydrogen

$$HCOOH \longrightarrow CO_2 + H_2$$

and presumably formic acid is the immediate precursor of hydrogen gas in most bacterial fermentations.[48]

The fermentation of sugars other than hexoses, organic acids, and alcohols results in the formation of acids and, sometimes, gases; but, for the most part, the mechanisms involved in these decompositions are unknown. Certain fragmentary observations are available, such as those of Kay,[49] who showed that when mannitol, glucose, glucuronic acid, glycuronic acid and saccharic acid are fermented by *Bacterium coli*, the yields of the more oxidized products (acetic and succinic acids) rise and those of the reduced product (ethyl alcohol) fall as one proceeds from the reduced substrate mannitol to the more highly oxidized saccharic acid. The fermentation of glycerol by this same organism has been found[50] to yield much the same end products as the fermentation of glucose. Similarly, the fermentation of the four-carbon acids, malic, tartaric and fumaric acids, by *Bacterium aerogenes* gives rise to the same products as the glucose fermentation;[51] the fermentation apparently proceeds via oxalacetic acid, which is decarboxylated to pyruvic acid.

The oxidation of ethyl alcohol to acetic acid through the agency of the acetic acid bacteria constitutes the so-called vinegar fermentation. Actually the process is not a fermentation in the usual sense but rather a specific partial oxidation and according to Quastel and Webley[52] is dependent upon the presence of thiamin. A variety of strictly aerobic forms, differing from one another to some degree but nevertheless closely related, have been described under the names of *Bacterium xylinum*, *Bacterium orleanense*, *Bacterium aceti*, *Bacterium pasteurianum* (not to be confused with the anaerobic nitrogen fixer, *Clostridium pastorianum*), *Bacterium ascendens*, etc. The oxidation may proceed directly

$$CH_3.CH_2.OH + O_2 \longrightarrow CH_3.COOH + H_2O$$

[48] Hydrogen may also arise in the decomposition of amino acids.
[49] Kay: Biochem. Jour., 1926, *20*:231.
[50] Braak: Diss. Delft, 1928.
[51] Barker: Kon. Akad. Weten. Amsterdam, 1936, *39*:1.
[52] Quastel and Webley: Biochem. Jour., 1941, *35*:192.

or by oxidation to acetaldehyde followed by a Cannizzaro reaction

$$\begin{array}{lllll} & & CH_3.CH_2OH & & CH_3.CH_2.OH \\ & & \nearrow \qquad \searrow & & \nearrow \\ CH_3.CH_2.OH \longrightarrow CH_2.CHO & & & CH_3.CHO & \qquad \text{etc.} \\ & & \searrow & & \searrow \\ & & CH_3.COOH & & CH_3.COOH \end{array}$$

Investigation has indicated that the oxidation of ethyl alcohol to acetic acid is but one of the oxidations these organisms are able to bring about; most of them are able to oxidize a number of alcohols to the corresponding acids.

Summary.—A number of facts become clear from this brief and superficial survey of the bacterial fermentation of sugars. Most obvious, perhaps, is the variety of mechanisms which appear to operate. Although a certain continuity runs through the biological oxidation of carbohydrates, bacteria are capable of a great number of variations upon this central theme—another manifestation of the physiological flexibility of these organisms. It is not possible, therefore, to develop a single scheme which will describe bacterial fermentations; each bacterial species constitutes a special case, and even a single organism may make use of more than one mechanism[53] and its fermentation processes are often an admixture of several mechanisms. Changes in environmental conditions which favor one type of decomposition over another are reflected in differences in the relative amounts of the various end products formed.[54]

It will be obvious, too, that all these reactions, with a few exceptions, involve the transfer of hydrogen by one or more mechanisms, a feature common and apparently fundamental to all these biological processes. The problem posed by these oxidations is not entirely one of the nature of the intermediate compounds making up the pathway of degradation but also one of the mechanisms of hydrogen transport, mechanisms that will undoubtedly prove to be highly complex.[55]

The efficiency of the heterotrophic bacteria in the utilization of energy made available through the oxidation of carbohydrates is difficult to determine with any great degree of accuracy, but available data indicate a somewhat higher efficiency than that of some of the autotrophic forms. It is probable that most of these organisms are able to make use of 25 to 35 per cent of the energy theoretically available to them.

The Hydrolysis of Proteins.—The ability to make use of amino acids is widespread among the bacteria, with the formation of compounds such as hydrogen sulfide, indol, amines and the like which are associated with the decomposition of proteins. The ability to hydrolyze native proteins to their amino acid constituents is, however, one that is possessed by relatively few kinds of bacteria. The limited occurrence of this capability is sufficiently marked that it is customary to speak of bacteria as fermentative or proteolytic in type according to whether carbohydrate oxidation or proteolysis characterizes their biochemical activities.

[53] See Wiggert and Werkman: Biochem. Jour., 1939, *33*:1061.
[54] See Gunsalas and Niven: Jour. Biol. Chem., 1942, *145*:131.
[55] Szent-Györgyi: Ztschr. physiol. Chem., 1937, *249*:211.

As compared with knowledge of the proteolytic enzymes of higher organisms, very little is known of the bacterial proteases.[56] In general they appear to be tryptic in nature, *i.e.*, they act in slightly alkaline media, whereas in protozoa and probably in fungi, pepsin-like enzymes occur. It is probable that a number of proteolytic enzymes are possessed by bacteria analogous to those occurring in higher organisms. Available evidence[57] indicates, for example, that decomposition may be carried to the polypeptide stage by one or more enzymes, while further dissolution is accomplished by means of another enzyme or group of enzymes.

The bacterial proteases are, for the most part, extracellular enzymes which are diffused into the medium and are present in sterile filtrates of cultures. Presumably protein molecules are too large to diffuse into the bacterial cell and are partially hydrolyzed outside. Split products probably diffuse into the cell to be further decomposed. Intracellular bacterial proteases have, however, been found.[58]

Of the bacterial enzymes the proteases appear to be the most markedly affected by the medium upon which the organisms producing them are grown. Chief among the factors influencing their production is the nitrogenous material present in the medium; in general, highly active filtrates may be obtained only from cultures in protein-containing media, although the detection of proteolytic activity in cultures grown in some of the so-called "synthetic" media containing no protein material indicates that the presence of substrate is not essential to the formation of the enzymes. The inorganic salts present are also of importance. It has been shown, for example, that the presence of magnesium and calcium salts is necessary for the production of gelatinase, the former stimulating growth but the latter actively stimulating the production of the enzyme. Undoubtedly a series of other environmental factors affect the production of proteolytic enzyme, but present knowledge is extremely fragmentary.

The ability to liquefy gelatin, a character of considerable value in the biochemical differentiation of bacteria, is often taken as indicative of the proteolytic potentialities of these organisms. Many organisms, however, which liquefy gelatin are unable to break it down further to amino acid units which may be used. *Staphylococcus aureus*, for example, although liquefying gelatin, is unable to hydrolyze the protein to abiuret substances and, presumably, can neither assimilate nor oxidize this substance. The digestion of gelatin by actively proteolytic organisms such as *Proteus vulgaris* is, on the other hand, carried rapidly to the amino acid stage, and evidences of the further decomposition of the amino acids, such as the liberation of ammonia, soon appear.

The resistance of pure native proteins, coagulated proteins and even purified proteose to attack by actively proteolytic bacteria in the absence of other food materials is well established.[59] Such bacteria, inoculated into a mineral salt solution containing a pure protein such as crystalline egg

[56] For a summary of present knowledge see Haines: Biol. Rev., 1934, *9*:235.
[57] Kendall and Keith: Jour. Inf. Dis., 1926, *38*:193.
[58] Avery and Cullen: Jour. Exp. Med., 1920, *32*:547, 571.
[59] Rettger: Jour. Bact., 1916, *1*:15.

albumin, do not multiply but in time die off. If, however, a small amount of peptone or meat extract is added to such a medium, the bacteria grow well and decompose the pure protein in a normal way. It is probable that extracellular protease inoculated with the bacterial cells is thereby so diluted that no hydrolysis takes place and, in the absence of smaller molecules that can diffuse into the cells and be broken down by enzymes concentrated there, the bacteria starve to death. Added food material such as peptone may be regarded simply as nutriment which makes possible the initiation of growth rather than as substances essential to the formation of proteolytic enzymes.

The evolution of ammonia which accompanies the bacterial decomposition of protein material is considerably curtailed if a fermentable carbohydrate is included in the protein-containing medium in which the organisms are grown. This phenomenon has been interpreted by Kendall[60] to indicate that carbohydrate exerts a "protein-sparing" effect on bacterial proteolysis analogous to its protein-sparing effect on mammalian metabolism. Precise work,[61] however, has shown that the analogy is spurious. A diminution in ammonia found might be due to either a decreased production or an increased utilization, since ammonia is the chief source of nitrogen for the great majority of bacteria. The presence of readily available energy in the form of a fermentable sugar stimulates growth with a coincident increase in ammonia assimilated and, in consequence, the ammonia present is diminished.

The Decomposition of Amino Acids.- As pointed out above, with very few exceptions, such as the autotrophic organisms, all bacteria are able to utilize amino acids, and in most cases the decomposition of amino acids present in an actively growing bacterial culture proceeds rapidly. The enzymes concerned in the primary utilization of amino acids have been studied at some length; present knowledge of them is reviewed by Gale.[62] Possibly these compounds may be used directly in some cases as building stones for bacterial protein; but in most instances some type of decomposition occurs which may liberate energy to the organism, and some of its products, such as ammonia, are the starting points of bacterial synthesis. Amino acids may be broken down in a variety of ways which may be summarized as follows:

(1) Hydrolytic decomposition which may result in the formation of a lower fatty acid and ammonia, of an alcohol, carbon dioxide and ammonia, or of an aldehyde, a lower fatty acid and ammonia by the following reactions

$$R.CH.NH_2.COOH + H_2O \longrightarrow R.CHOH.COOH + NH_3$$
$$R.CH.NH_2.COOH + H_2O \longrightarrow R.CH_2.OH + CO_2 + NH_3$$
$$R.CH.NH_2.COOH + H_2O \longrightarrow R.CHO + H.COOH + NH_3$$

which are often brought about by aerobic organisms. The decomposition to an alcohol is frequently brought about by bacteria.

[60] Kendall: Jour. Inf. Dis., 1922, *30*:311.
[61] Waksman and Lomanitz: Jour. Agr. Res., 1925, *30*:263.
[62] Gale: Bact. Rev., 1940, *4*:135.

(2) Decarboxylation with the formation of the corresponding amine (the so-called ptomaines including pentamethyldiamine or cadaverine from lysine and tetramethylenediamine or putrescine from ornithine or arginine) is often brought about by obligate anaerobes

$$R.CH.NH_2COOH \longrightarrow R.CH_2.NH_2$$

Similarly, decarboxylation of a monoamino dicarboxylic acid will result in the formation of a monocarboxylic acid as in the formation of β-alanine from aspartic acid.

(3) Reductive deamination with the formation of saturated fatty acids and ammonia

$$R.CH.NH_2.COOH + H_2 \longrightarrow R.CH_2.COOH + NH_3$$

or

$$R.CH.NH_2.COOH + H_2 \longrightarrow R.CH_3. + NH_3 + CO_2$$

with the formation of the saturated hydrocarbon. These reactions are brought about by anaerobic bacteria which may, for example, decompose glycine to acetic acid and ammonia, or methane, carbon dioxide and ammonia.

(4) Deamination and desaturation at the α-β linkage with the formation of the unsaturated fatty acid and ammonia

$$R.CH_2.CH.NH_2.COOH \longrightarrow R.CH{=}CH.COOH + NH_3$$

is also carried out by anaerobic bacteria.

(5) Oxidative deamination with the formation of ammonia, carbon dioxide and a fatty acid of one less carbon is brought about by aerobic organisms

$$R.CH.NH_2.COOH + O_2. \longrightarrow R.COOH + NH_3 + CO_2$$

(6) Anaerobic oxidative deamination accompanied by the evolution of hydrogen and the disruption of the amino acid molecule has been described recently. The products of decomposition of glutamic acid have been determined

$$5\ C_5H_9O_4N + 6\ H_2O \rightarrow 6\ CH_3.COOH + 2\ CH_3(CH_2)_2.COOH + 5\ CO_2 + H_2 + 5\ NH_3$$

The reaction probably proceeds by stages which are as yet unknown. This type of decomposition is sometimes known as the "fermentation" of amino acids.

(7) The mutual oxidation and reduction of pairs of amino acids resulting in deamination and decomposition, undoubtedly an important source of energy to some obligate anaerobes and possibly facultative and aerobic organisms as well. Glycine, for example, is reduced to acetic acid and ammonia, and alanine oxidized to acetic acid, carbon dioxide and ammonia

$$CH_3.CH.NH_2.COOH + \tfrac{1}{2}\ O_2 \longrightarrow CH_2.COOH + NH_3 + CO_2$$
$$CH_2.NH_2.COOH + H_2 \longrightarrow CH_3.COOH + NH_3$$

The reactions are, of course, an oxidative and a reductive deamination respectively, but the significance lies in the fact that one amino acid may act as hydrogen acceptor in the oxidation of another.

The aromatic amino acids are usually attacked first at the side chain, while the ring may or may not be broken. A number of organisms decarboxylate histidine to the physiologically active substance histamine and carry the decomposition no further. The breakdown of tryptophane to indol by some bacteria and not by others is taken advantage of in the biochemical differentiation of many species from one another. Tyrosine may be decomposed to phenol or broken down completely, and a number of organisms break the pyrrolidine ring of proline without difficulty. Owing to differences in ring structure, the decomposition of the aromatic amino acids becomes a series of special cases which cannot be considered further here.

The types of amino acid breakdown outlined above represent a generalization. No single amino acid has as yet been shown to undergo all of the decompositions nor is any single bacterial species capable of bringing about all of these transformations. On the other hand, the bacterial decomposition of an amino acid often involves not a single type of breakdown alone but a series of reactions consisting of several of these types. The nature of the products formed is dependent, to some degree, upon oxygen tension; some compounds such as hydroxy acids may be formed under aerobic conditions but are unstable under anaerobic conditions. The *p*H of the medium is also of considerable importance, in some cases at least.[63] The fact that ammonia is almost always liberated is of considerable significance, for ammonium salts are utilized as a source of nitrogen by the great majority of bacteria.

Aerobic and anaerobic protein decompositions are often differentiated by the terms decay and putrefaction. Superficially there would appear to be some justification for regarding the two as separate processes the justification being largely a matter of the unpleasant odors associated with the reduced products of anaerobic decomposition as contrasted with the lack of odor of the oxidized end products of the aerobic decomposition. Such a distinction has no reality, however, for the processes of decomposition are essentially the same in both cases. Both involve preliminary hydrolysis to amino acids and have further breakdown mechanisms in common. Oxidative deamination, for example, is commonly associated with aerobic organisms, but it also occurs in the oxidation-reduction of pairs of amino acids, a reaction brought about by many of the obligate anaerobes.

The Decomposition of Fats.—From the quantitative point of view fats are not an important food source for bacteria. Bacterial lipases are not uncommon, however, and the action of bacteria on fat-containing media has some use as a differential character. Following hydrolysis the glycerol is readily fermented by a wide variety of bacteria but the ability to decompose the higher fatty acids does not seem to be common and a number of these, such as palmitic, stearic, oleic and other acids, cannot be utilized as a source of carbon by many of the well-known bacteria. The oxidative decomposition of fatty acids assumes some importance with regard to the development of rancidity of fat-containing foods.[64]

[63] *Cf.* Gale: Biochem. Jour., 1940, *34:*853.
[64] Jensen and Grettie: Food Res., 1937, *2:*97.

NITROGEN FIXATION[65]

The ability to use molecular nitrogen as a nutrient is, like the oxidation of ammonia and nitrite, a property of a limited number of bacterial species. Evidence of the biological basis of nitrogen fixation was reported in 1862,[66] and it was later shown that the increase in organic nitrogen content of uncultivated soils could be prevented by sterilization or storing at a low temperature. Following the work of Winogradsky, Beijerinck, Hellriegel and Wilfarth and others it became apparent that atmospheric nitrogen is fixed by

(1) free-living bacteria both
 (a) anaerobic and
 (b) aerobic and by
(2) bacteria living symbiotically with leguminous plants.

The anaerobic organism, *Clostridium pastorianum*, isolated by Winogradsky,[67] was the first nitrogen-fixing bacterium to be studied in pure culture. When cultivated under anaerobic conditions in a medium containing glucose but no nitrogen, the organism grows well, obtaining its energy through the fermentation of the sugar and its nitrogen from nitrogen gas. The amount of nitrogen fixed is proportional to the amount of glucose fermented—2.4 to 2.9 milligrams of nitrogen fixed per gram of glucose fermented in Winogradsky's experiments. The fixation of nitrogen is inhibited by the presence of ammonium salts in the medium and may be lost through continued cultivation on nitrogen-containing media. *Cl. pastorianum* is a spore-forming rod closely related to the bacteria of the so-called amylobacter group (so-called because many of them stain blue with iodine), organisms which ferment carbohydrate to butyric acid. The chief products of the fermentation of glucose by *Cl. pastorianum* are acetic and butyric acids and carbon dioxide and hydrogen. It has been found[68] that the ability to fix nitrogen may be "restored" to some amylobacter species by a method of "soil passage" which restores this property to strains of *Cl. pastorianum* which have lost it through prolonged cultivation on laboratory media. It is probable that all these organisms constitute a relatively homogeneous group whose natural habitat is soil.

The aerobic nitrogen-fixing bacteria were discovered by Beijerinck in 1901[69] and named by him *Azotobacter*. Two species were originally suggested: *Azotobacter chroococcum*, a pigmented (deep brown to black), nonmotile organism found predominantly in soil; and *Azotobacter agilis*, a motile form producing a soluble green pigment and found in water. Two other species have been found[70] in this country, *Azotobacter vinelandii* and *Azotobacter beijerincki*. Although one or two additional species have been described, these four have, until recently, been the generally recognized members of the genus, with *Az. chroococcum* as typical of the group. This

[65] Biochemical nitrogen fixation is reviewed by Burk and Burris: Ann. Rev. Biochem., 1941, *10*:587.
[66] Jodin: Compt. Rend. Acad. Sci., Paris, 1862, *55*:612.
[67] Winogradsky: Compt. Rend. Acad. Sci., Paris, 1893, *116*:1385.
[68] Bredemann: Centralbl. f. Bakt., 1909, Abt. II, *23*:385.
[69] Beijerinck: Centralbl. f. Bakt., 1901, Abt. II, *7*:561.
[70] Lipman: N. J. Agr. Station Ann. Rept., 1903, *24*:217; *ibid.*, 1904, *25*:237

species has a world-wide distribution; the other three are apparently more restricted, although *Az. agilis* may have a wider distribution than formerly thought. All fix nitrogen in alkaline environments, fixation proceeding most rapidly at pH 7.0 to 7.5. Recently, however, Starkey[71] has reported the isolation of a species designated as *Az. indicum* from acid soil which fixes nitrogen when grown in an acid (pH as low as 3.0) medium. *Azotobacter* survives for long periods of time, as long as twenty years, and still is able actively to fix nitrogen.[72]

In addition to reaction, a number of other factors govern nitrogen fixation by these organisms. Phosphate, calcium and oxidizable carbon compounds (mannitol or propionate are usually supplied in laboratory media), in addition to certain metals such as iron and molybdenum,[73] are necessary for fixation, and the numbers of these organisms in a given soil may often be increased by the addition of chalk, lime, phosphate or carbohydrate, depending upon the deficiencies of the particular soil.

Fig. 16.—*Azotobacter chroococcum.* Three-day culture on nitrogen-free dextrose medium. Nigrosin relief preparation; × 978 (Starkey).

Like *Cl. pastorianum, Azotobacter* does not fix nitrogen in the presence of adequate amounts of nitrogen compounds in the medium but, unlike the anaerobe, does not lose the ability to fix nitrogen through continued cultivation on nitrogen-containing media. Although *Azotobacter* fixes nitrogen in the free-living state, it is not infrequently found in nature living symbiotically with higher plants. *Chlorella*, for example, may grow in association with the bacterium, the former assimilating carbon dioxide and the latter molecular nitrogen.

The mechanism by which nitrogen is fixed is obscure. Winogradsky suggested that *Cl. pastorianum* might bring about a direct combination of nitrogen with nascent hydrogen liberated in the fermentation of glucose to form ammonia. It has been generally supposed that the fixation of ni-

[71] Starkey: Science, 1939, *89*:267. See also Starkey and De: Soil Sci., 1939, *47*:329.
[72] Greaves and Jones: Soil Sci., 1941, *52*:359.
[73] *Cf.* Burk: Ergebnisse der Enzymforschung, 1934, *3*:23.

trogen is an endothermic process, but Burk[74] has pointed out that fixation might take place by means of exothermic reactions either in the presence of hydrogen arising from the fermentation of glucose or formate or as an oxidation in the presence of free oxygen.[75] No intermediate compounds have been detected, however, and fixed nitrogen appears only in the form of bacterial protoplasm. Nitrogen in other forms such as ammonia has been found to result from autolysis of the bacterial cells.

The efficiency of the fixation process is variable depending upon the partial pressure of oxygen. The high respiratory rate of *Azotobacter*, QO_2 = 2000 to 4000, the highest recorded for any living cell, is at a maximum at 10 to 15 per cent oxygen, falling off sharply on either side of this optimum. Maximum nitrogen fixation, however, takes place at 4 to 5 per cent oxygen, and the efficiency of the fixation process increases with decreasing oxygen pressure. Under optimum conditions nitrogen is fixed with an efficiency of approximately 11 per cent of the maximum theoretically attainable, a figure which falls off to about 1 per cent under atmospheric conditions.

Fig. 17.—Colonies of *Rhizobium radicicola* on nutrient agar. Twenty-four hour culture; × 3.

The Symbiotic Nitrogen-Fixing Bacteria.—The enrichment of a soil by the cultivation on it of leguminous plants such as soy beans, vetch, peas, clover and the like has been known throughout recorded history. Although such plants were early (1838) shown to take up atmospheric nitrogen, the relation between nodule formation and nitrogen fixation was first conclusively demonstrated by Hellriegel and Wilfarth[76] and the bacteria present in the nodules were cultivated by Beijerinck.[77] These organisms, called by him *Bacillus radicicola* and now known under the generic

[74] Burk: Jour. Gen. Physiol., 1927, *10*:559.

[75] An oxidation to HNO_3. If the concentration of HNO_3 is less than 0.1 molar, $\triangle F$ has a positive value.

[76] Hellriegel and Wilfarth: Beilageheft Ztschr. ver. Rübenzuckerind, 1888.

[77] Beijerinck: Bot. Ztg., 1888, *46*:725, 741, 758, 782, 797.

name of *Rhizobium*, were shown through subsequent work to fix atmospheric nitrogen when living symbiotically in the root nodules. Although the bacteria occur free in the soil, nitrogen is not fixed except in intimate association with the host plant.

The bacteria in pure culture on artificial media are small, motile rods which do not form spores. They show a high degree of pleomorphism in the root nodules, however; small oval forms and branching forms (bacteroides) are found admixed with the rod forms. Some workers regard these bacteria as a single species, *Rhizobium leguminosarum*, which is made up of a variety of strains differing from one another immunologically and in their ability to infect the various species of leguminous plants. These strains fall into two groups, however, one having peritrichous flagella and being somewhat more active biochemically than the other, which has monotrichous flagella. The separation of two species, *Rhizobium leguminosarum* and *Rhizobium radicicola*, has been made on the basis of the legumes

Fig. 18.—*Rhizobium radicicola.* Smear from a pure culture; fuchsin; × 1050.

which they can infect. Possibly these groups constitute legitimate species; some regard them as such while others consider them only as strains of the single species. As a group they appear to be closely related to *Bacterium aerogenes*, Friedländer's bacillus and similar organisms. An organism known as *Bacterium radiobacter* is often found associated with the nodule bacteria but does not fix nitrogen.[78]

In spite of difficulties arising from the fact that the fixation of nitrogen by *Rhizobium* occurs only in association with the host plant, the mechanism of fixation is somewhat better understood than in the case of the free-living nitrogen fixers, chiefly through the work of Virtanen and his colleagues.[79] It has been found that not inconsiderable quantities of nitrogen are excreted by the root nodules during the early stages of growth

[78] For a recent study of this organism see Hofer: Jour. Bact., 1941, *41*:193.

[79] *Cf.* Virtanen and Laine: Nature, 1938, *142*:165; Wilson: *The Biochemistry of Symbiotic Nitrogen Fixation.* University of Wisconsin Press. Madison. 1940.

in the form of aspartic acid and β-alanine together with a small quantity of the oxime of oxalacetic acid. It appears that nitrogen is fixed as hydroxylamine, which in turn condenses with oxalacetic acid supplied by the plant. The oxime is then reduced to aspartic acid, which serves as a starting material for the synthesis of other amino acids. The β-alanine found presumably arises through decarboxylation of the aspartic acid. The course of the fixation process may be expressed thus:

$$\begin{array}{lll}
N_2 \longrightarrow\ ?\ \longrightarrow NH_2OH & & \\
 & \searrow & \\
 & & COOH.C(NOH).CH_2.COOH \longrightarrow \\
 & & \qquad COOH.CHNH_2.CH_2.COOH \\
 & \nearrow & \\
C_6H_{12}O_6 \longrightarrow COOH.CO.CH_2.COOH & &
\end{array}$$

Plants other than the *leguminosae* show nodule formation which, in some cases, is associated with nitrogen fixation. A number of species of alder (*Alnus*), for instance, have root nodules and there is evidence[80] that nitrogen fixation occurs. Nodule formation is not confined to roots: some plants, the *Rubiaceae* and *Dioscorea*, show bacteria-containing nodules on the leaves, and in one instance these have been shown[81] to fix atmospheric nitrogen.

It is open to question whether bacteria other than those described above are able to fix nitrogen. Fixation has been reported for a variety of organisms such as the pneumococcus, but the evidence is inconclusive and fragmentary. It has been shown[82] recently, however, that the blue-green alga *Nostoc* is able to fix atmospheric nitrogen.

THE NUTRITIVE REQUIREMENTS OF BACTERIA[83]

It is already apparent that bacteria as a group include a wide variety of physiological types, ranging from the photosynthetic and autotrophic forms to those which metabolize carbohydrates and organic nitrogenous substances by means of complex enzyme systems. The food requirements of these organisms are equally diverse, some being able to utilize inorganic compounds of carbon and nitrogen while others require organic compounds of varying degrees of complexity. The kinds of food materials that must be supplied to an organism may be considered to fall into three categories:

(1) compounds that can be oxidized by the organism and thereby serve as sources of energy;

(2) compounds that serve as building stones or as precursors to building stones in the synthesis of bacterial protoplasm; and

[80] Hiltner: Lanw. Vers. Sta., 1896, *46*:153. For more recent work see Plotho: Arch. f. Mikrobiol., 1941, *12*:1.

[81] van Faber: Jahrb. wiss. Bot., 1912, *51*:283; *ibid.*, 1914, *54*:243.

[82] Allison, Hoover and Morris: Bot. Gaz., 1937, *98*:433.

[83] The literature to 1936 has been reviewed by Knight: Medical Research Council Special Report Series, No. 210, 1936; and to 1938 by Lwoff: Ann. Inst. Pasteur, 1938, *61*:580.

(3) substances which, for want of a better name, are designated as growth accessory substances or vitamin-like substances and which, in many cases, appear to function as components of catalytic systems in the economy of the organism.

Although such a classification of food materials is convenient for purposes of exposition, sharp distinctions are sometimes difficult to make, as will appear.[84]

In general, the utilization of a given substance as a food by an organism is dependent upon two factors. One, perhaps the more obvious, is the possession by the organism of enzyme systems which make possible the decomposition or assimilation of the substance in question. Cellulose, for example, is not a food for organisms which are unable to decompose it. The second factor is the concentration of the substance, which may vary from a lower limit below which the bacterium is unable to grow to an upper limit beyond which additional material is simply excess. Very high concentrations may actually be toxic, *viz.*, the preservation of foods in syrups. Butterfield[85] has shown that between these two limits a regular relation exists between the amount of bacterial growth and the concentration of nutrient material; if the logarithms of the maximum numbers of bacteria are plotted against the logarithms of the concentrations of food, the points fall on a straight line. The maximum numbers of bacteria are, in turn, dependent upon the size of the individual organisms, the relation between size and numbers of individuals being inverse the smaller the bacterium the greater the number of individual cells. When the volume of the cells is taken into consideration, however, it appears that a given volume of bacterial substance is formed in a given concentration of nutrient, the larger numbers of small organisms being but a consequence of wrapping the same amount of protoplasm in smaller packages.

Oxidizable Substances.—The compounds that may serve as sources of energy to the growing bacterial culture have already been discussed in connection with the mechanisms of oxidation used by these organisms. Suffice it to say that the autotrophic forms require as food material the inorganic compounds of nitrogen, sulfur and iron that they can oxidize, and that such compounds may be regarded as nutriment in the same way that carbohydrates and amino acids are regarded as foodstuffs for the heterotrophic forms. The autotrophic bacteria, however, exhibit a high degree of specificity with respect to oxidizable substances which has no counterpart in the metabolism of the heterotrophic microorganisms. The latter, both as a group and as individual species, may obtain energy from a great variety of organic compounds of carbon and nitrogen. As long as such compounds are susceptible to attack by the enzyme systems of the bacterium, they may serve as food materials.

"Building Stones."—The second group of food substances, those that serve as building stones, may be considered as sources of the chemical

[84] For a critical discussion of groups (2) and (3) see Burrows: Quart. Rev. Biol., 1936, *11*:406.

[85] Butterfield: Pub. Health Repts., 1929, *44*:2865.

elements which, in appropriate and often highly complex combinations, make up the bacterial protoplasm. Chief among these elements are carbon and nitrogen which the organism may be able to assimilate in a variety of forms. The ability to assimilate inorganic carbon in the form of carbon dioxide or carbonates is characteristic of the autotrophic bacteria. The heterotrophic microorganisms require organic compounds as sources of carbon but do not appear to be particularly fastidious as to the nature of these compounds as long as they may be attacked by the bacterial enzymes. An organism may, for example, assimilate some of the carbon of a fermentable sugar[86] or it may grow normally in the presence of one or more amino acids in mineral salt solutions. The apparent relative unimportance of the nature of the carbon compound suggests that bacteria may break down all manner of carbon compounds to one or more substances which they use as a starting material for the synthesis of cell substance. There is some evidence[87] that pyruvic acid may be such a starting point, and possibly other simple carbon compounds may be assimilated directly.

Bacteria as a group exhibit a striking uniformity in their ability to use ammonia or ammonium salts as sources of nitrogen. Not only the autotrophic forms but even the most fastidious of the heterotrophes use nitrogen in this inorganic form. Although it is not unlikely that some amino acids are assimilated directly by some bacteria, the great majority attack these compounds and break them down. One of the early steps in the decomposition is deamination, hydrolytic, reductive or oxidative, with the production of free ammonia, a part of which the organism then takes up and presumably makes use of in the synthesis of amino acids which are, in turn, condensed to form protein molecules.

The Essential Amino Acids.—Experimental evidence of recent years is strongly suggestive of the need on the part of some bacteria not only for the elements carbon and nitrogen, but also for these elements in the form of preformed molecular structures. It has been pointed out[88] that the failure of certain bacteria to grow in mineral salt solutions containing fermentable carbohydrate and ammonia might be accounted for on the assumption that such organisms lacked the ability to synthesize certain molecular structures, such as those contained in certain amino acids, and, in consequence, such structures might have to be supplied preformed. The basic assumption is, of course, analogous to the concept of "essential" amino acids of mammalian nutrition. A considerable quantity of evidence indicates that a number of bacterial species require the presence of certain amino acids in order to grow in mineral salt solutions. One strain of the diphtheria bacillus, for example, has been found to require a mixture of tryptophane, cystine, methionine, valine, histidine, glycine and glutamic acid, while another requires leucine, methionine, valine and glutamic acid. *Clostridium botulinum* requires cystine, leucine, lysine, glycine and proline, and another anaerobe, *Clostridium sporogenes*, requires tryptophane, cys-

[86] For example, see Clifton: Jour. Bact., 1939, *37*:523.

[87] Quastel: Biochem. Jour., 1925, *19*:641. Also Fromageot and Safavi: Enzymologia, 1939, *6*:57.

[88] Burrows: Jour. Inf. Dis., 1933, *52*:126.

tine, leucine, methionine, valine, tyrosine, histidine, arginine and phenylalanine. The amino acid requirements of streptococci are similarly complex. The requirements of some other organisms are not so elaborate; some strains of the typhoid bacillus, for instance, will grow in a mineral salt solution plus tryptophane, while others need no amino acids. The significance of observations such as these is not altogether clear at the present time. It is assumed, of course, that such required amino acids are directly assimilated as building stones which the organism is incapable of synthesizing as indicated above; but the demonstration of the oxidation-reduction of pairs of amino acids by obligate anaerobes indicates that these compounds may sometimes function as energy-yielding compounds rather than building stones. Furthermore, analytical evidence[89] suggests that the apparent necessity for the inclusion of amino acids in mineral salt solutions may not be indicative of a lack of synthetic abilities on the part of the microorganisms.

Other evidence pointing in this same direction and indicating that the question of required amino acids may become quite complex has been found by Gladstone.[90] He observed that the anthrax bacillus could be grown in an amino acid medium including, among others, leucine, isoleucine and valine. Omission of any one greatly reduced or prevented growth, indicating their essential nature. If, however, all three were omitted growth occurred and any single one was toxic. A definite antagonism among amino acids was thus indicated and further demonstrated; a competitive blocking of enzyme surfaces was offered in explanation.

The Essential Elements.—Elements other than carbon and nitrogen are a part of the bacterial protoplasm and must, of necessity, be supplied in a culture medium. Oxygen is, of course, contained in carbohydrates and amino acids as well as available in the gaseous form to all but the obligate anaerobes. Of the other elements, phosphorus predominates quantitatively because of the high nucleic acid content of the cells. It is generally supplied in the form of phosphate and probably may be assimilated by the organism in the form of other inorganic compounds. A number of other elements, such as iron, magnesium, calcium, etc., have been shown to be necessary to bacterial growth, and certain organisms may have special requirements as in the case of *Azotobacter*, which requires the presence of elements such as molybdenum and vanadium for nitrogen fixation (which in this case is growth, since the nitrogen is fixed as bacterial protoplasm). It has been found also that iron concentration is of paramount importance in toxin formation by the diphtheria bacillus, the optimum being 0.14 micrograms per cubic centimeter.[91] Similar results have been obtained in studies of the formation of tetanus toxin.[92] Probably a great number of chemical elements go to make up the bacterial cell substance and are, therefore, necessary parts of the food supply; but in the great majority of cases they are re-

[89] Burrows: Jour. Inf. Dis., 1939, *64*:145.
[90] Gladstone: Brit. Jour. Exp. Path., 1939, *20*:189.
[91] Pappenheimer and Johnson: Brit. Jour. Exp. Path., 1936, *17*:335; Pappenheimer: *ibid.*, 1936, *17*:342.
[92] Mueller and Miller: Proc. Soc. Exp. Biol. Med., 1940, *43*:389.

quired only in the minute traces with which many chemical compounds of the highest purity are contaminated. Obvious technical difficulties have prevented intensive study, but it is quite likely that all of these "biologically rare" elements may be assimilated in the form of inorganic compounds.

Growth Accessory Substances or Bacterial Vitamins.—The third group of food materials, the growth accessory substances, consists of organic compounds which are necessary to growing bacteria only in minute amounts. Presumably these substances cannot be synthesized by the cell, thereby resembling the essential amino acids but differing from them in that the growth accessory substances apparently function in the cell economy as catalysts rather than building stones for cell substance proper. The search for such substances goes back over many years, even antedating the discovery of the vitamins of mammalian physiology. The great majority of investigations were based upon a general type of experiment in which a solution which did not support the growth of the bacterium under consideration was supplemented by extracts of various plant and animal tissues. Since many of these extracts permitted growth in the otherwise inadequate solution, it was assumed that they contained active substances which were variously termed biocatalyzers, growth hormones and the like. The interpretation of experiments carried to this point but no further is exceedingly difficult. In very recent years, however, more precise information regarding the nature of these substances has become available.

It is of particular interest that the majority of these substances have proved to be identical with various components of the vitamin B complex, suggesting a close relation between the metabolic processes of organisms as diverse as bacteria on the one hand and mammals and other higher animals on the other. It is not inappropriate, therefore, to refer to these substances as *bacterial vitamins*. Other substances required by bacteria and included in this category are glutamine, purines such as adenine or uracil, pimelic acid, and others. The requirements of a number of bacteria have been worked out in some detail[93] but it should be noted that there may be, and often is, considerable variation from strain to strain of the same species.

The first bacterium to be studied in detail in this respect was the diphtheria bacillus which Mueller[94] found to require nicotinic acid and β-alanine, the latter presumably serving as a precursor of pantothenic acid; some strains require pimelic acid. *Staphylococcus aureus* was similarly studied by Knight[95] and found to require nicotinic acid and thiamin and, for anaerobic growth, pyruvic acid and uracil had to be supplied. It has been found by others that certain strains require biotin. Similarly, the lactic acid bacteria have been found to require riboflavin, pantothenic acid, nicotinic acid, pyridoxine and other as yet unidentified substances, and the hemolytic streptococci must be supplied with pantothenic acid, pyridoxine, thiamin, nicotinic acid, glutamine and certain purines.

[93] The nutritive requirements of individual bacterial species are considered in later chapters.

[94] See the reviews by Mueller: Jour. Bact., 1938, *36*:499; Bact. Rev., 1940, *4*:97.

[95] Knight: Biochem. Jour., 1937, *31*:731, 966; *ibid.*, 1938, *32*:1241.

In other instances precise study has made possible an understanding of growth requirements already known. Thus, in the case of the hemophilic organism, the influenza bacillus, it has long been known that two substances present in fresh blood media are required. One of these, the "X factor," is

B COMPLEX VITAMINS REQUIRED BY VARIOUS SPECIES OF BACTERIA*

Bacterium	Substance Required in Micrograms per cc. of Medium							
	Thia-	Ribo-flavin	Panto-thenic Acid	Nico-tinic Acid	Biotin	Pyri-dox-ine	Folic Acid	*p*-Amino-benzoic Acid
Staphylococcus aureus	0.003			0.20				
Streptococcus hemolyticus C203S	0.001	0.004	1.00	0.10		2.00		
Streptococcus epidemicus X40		0.10	0.50					
Streptococcus hemolyticus D-NYS			1.25					
Streptococcus zymogenes						0.50		
Streptococcus lactis R							0.0005	
Pneumococci			1.00					
Shigella dysenteriae				0.10				
Proteus morganii			0.20					
Brucella melitensis	0.02							
Lactobacillus casei		0.04	0.03	0.10	0.0001	0.06	0.00005	
Lactobacillus arabinosus 17–5				0.10	0.00015			0.0002
Clostridium tetani			0.05					
Corynebacterium diphtheriae				1.00				

* Data from various authors; modified from table of Stokes, Gunness and Foster: Jour. Bact., 1944, *47*:293.

heat-stable and associated with hemoglobin, while the other, the "V factor," is heat-labile and found in yeast and various vegetable extracts as well as in blood. It is now known that the X-factor may be replaced by hematin and the V factor by coenzyme I or II.

Other bacteria, however, have much simpler nutritive requirements.

The typhoid bacillus, for example, does not require added growth accessory substances; most strains of dysentery bacilli and *Proteus* require only nicotinic acid.

These nutritive requirements are generally believed to be an expression of limitations in the synthetic abilities of the microorganisms, the assumption being the same as that first postulated for the essential amino acids,[88] *viz.*, the substance in question is an essential metabolite which cannot be synthesized by the microorganism and therefore must be supplied as such. From this point of view there appear to be varying degrees of limitation. For example, the influenza bacillus requires the entire cozymase molecule but most bacteria require only nicotinic acid or nicotinamide, and given this structure presumably synthesize the remainder of the molecule. The case of thiamin is somewhat similar. The molecule consists of pyrimidine and thiazole (see structural formula), loosely joined. *Staphylococcus aureus* is apparently unable to synthesize either ring, but grows in the presence of both pyrimidine and thiazole and is presumably able to form the complete thiamin molecule from its two component parts. Other organisms, such as the influenza bacillus, require the presence of the complete molecule, while still others will grow in the presence of one or the other ring structure, supposedly synthesizing the one not required and joining the two to form the thiamin molecule.

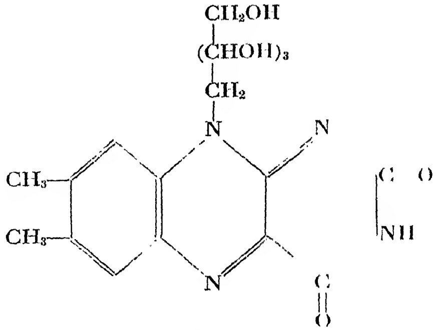
The function of bacterial vitamins in the physiologic economy of the organism is of particular importance but has not as yet been studied extensively. It was early predicted[84] that they have a catalytic function and present knowledge of their nature appears to substantiate this. The significance of riboflavin and thiamin as the functional, prosthetic groups of the respiratory enzymes flavoprotein, or yellow enzyme, and cocarboxylase respectively will be readily apparent (p. 84). Similarly, the rather general requirement for nicotinic acid is significant since this is a part of the pyridine nucleotide (DPN and TPN) molecules. It is generally believed, therefore, that these substances are precursors of respiratory enzymes, representing the part of the molecule the organism is unable to synthesize, which are essential to the metabolism of the cell. This is supported by a certain amount of experimental evidence. For example, the addition of thiamin to thiamin-deficient cells of *Staphylococcus aureus* and the propionic and lactic acid bacteria results in a marked increase in the rate of carbon dioxide production from pyruvate, and in the case of the last two, sugar and lactate breakdown are somewhat increased.[96] Similarly, methylene blue reduction and oxygen uptake by deficient dysentery bacilli are stimulated by the addition of nicotinic acid or its amide, or diphosphopyridine nucleotide.[97] Such experiments must be interpreted with caution, however. For example, Mann and Quastel[98] have demonstrated the presence of a hydrolytic enzyme, coenzyme I-nucleotidase, in fresh brain tissue

[96] Hills: Biochem. Jour., 1938, *32:*383; Silverman and Werkman: Jour. Bact., 1939, *38:*25; *ibid.*, Proc. Soc. Exp. Biol. Med., 1939, *40:*369.

[97] Dorfman and Koser: Jour. Inf. Dis., 1942, *71:*241.

[98] Mann and Quastel: Biochem. Jour., 1941, *35:*502.

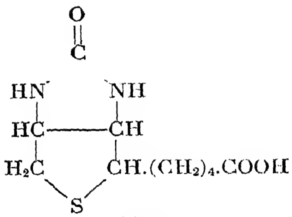
which is responsible for the breakdown of the coenzyme, and which can be completely inhibited by high concentrations of nicotinamide. The stimulating effect of nicotinamide on lactic dehydrogenase activity of



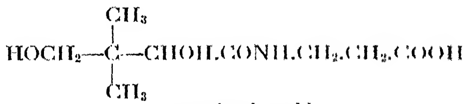
riboflavin

COOH.CHNH$_2$.CH$_2$.CH$_2$.CONH$_2$

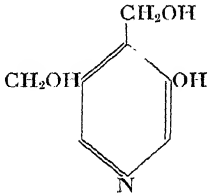
glutamine



biotin

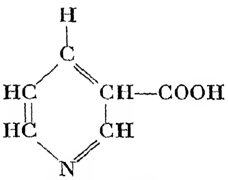


pantothenic acid

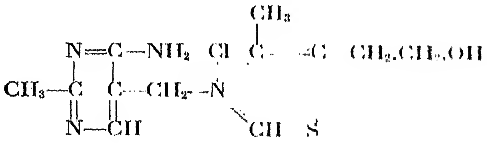


pyridoxine

p-*aminobenzoic acid*



nicotinic acid



pyrimidine *thiazole*

thiamin

tissues in the presence of the nucleotidase is, therefore, not a result of synthesis of nicotinamide to the coenzyme, but due to a competition of nicotinamide with the coenzyme for the nucleotidase.

In general, though, it is not unreasonable to assume that these substances are associated with the catalytic decomposition of carbon compounds and may be considered as parts of the energy-yielding mechanisms of the cell rather than units used in the building up of cell substance proper. Whether other substances, such as glutamine and *p*-aminobenzoic acid, function in this way is as yet unknown; it is possible that in some cases at least they may be associated with the synthesis of the protoplasmic structure of the cell.

Carbon Dioxide.—The intimate relationship between carbon dioxide tension and bacterial multiplication has long been known. The gonococcus, meningococcus, and *Brucella abortus*, for example, require appreciable partial pressures of the gas for growth, especially in primary isolation, and are usually incubated in vessels containing perhaps 10 per cent carbon dioxide. It is also required by other bacteria, possibly all, for growth though in very much smaller amounts. This may be demonstrated by gassing freshly inoculated cultures with CO_2-free air; if the aeration is carried on vigorously no growth occurs, but if aeration is discontinued it begins. Once growth has been initiated, however, it is not possible to stop it by aeration; presumably carbon dioxide is formed by the metabolizing cells sufficiently rapidly that it cannot be completely washed out in this way. The earlier literature has been reviewed by Valley[99] who suggests that a carbon dioxide tension is essential for cell division, but the supporting evidence for this is only circumstantial. The apparent mutually stimulating effects of allelocatalysis (p. 53) have been accounted for by some workers on the basis of carbon dioxide production. It has been shown, however, by Hes[100] that carbon dioxide exerts a catalytic effect on certain oxidation-reduction reactions, the reduction of methylene blue by bacterial suspensions being greatly retarded in its absence. Some purely hypothetical explanations have been advanced, such as the postulation of intracellular *p*H effects.

Carbon dioxide appears to influence the physiologic processes of bacteria in still other ways; it has been found, for example, that certain strains of staphylococci produce increased amounts of hemolysin[101] and enterotoxic substance[102] when cultured in the presence of 10 to 20 per cent carbon dioxide. The presence of the gas also appears to affect nutritive requirements in some instances; hemolytic streptococci require adenylic acid or other purines when the tension is low (less than 2 mm. Hg) but dispense with these substances when the tension is high (20 mm. Hg).[103]

The Heterotrophic Assimilation of Carbon Dioxide.—The demonstration by Werkman and his co-workers[104] of the heterotrophic assimilation of carbon dioxide has clarified considerably the relation of carbon dioxide to

[99] Valley: Quart. Rev. Biol., 1932, *3*:209.

[100] Hes: Nature, 1938, *141*:647. See also a general discussion by this worker in Ann. Fermentations, 1938, *4*:547.

[101] Burnet: Jour. Path. and Bact., 1929, *32*:717; *ibid.*, 1930, *33*:1.

[102] Woolpert and Dack: Jour. Inf. Dis., 1933, *52*:6.

[103] Pappenheimer and Hottle: Proc. Soc. Exp. Biol. Med., 1940, *44*:645.

[104] See the reviews by Werkman and Wood: Bot. Rev., 1942, *8*:1; and by Werkman: Advances in Enzymology, 1942, *2*:135.

the metabolic processes of bacteria. Werkman has pointed out that one of the differences between autotrophic and heterotrophic bacteria may now be defined with some precision, *i.e.*, the autotrophes are able to bring about a carbon-to-carbon linkage when both carbons are inorganic, while the heterotrophes can, in general, link carbon to carbon only when one or both of the carbons is organic.

This was first demonstrated in the formation of succinic acid from glycerol by fermentation with propionic acid bacilli, and by the use of heavy carbon and radioactive carbon it has been shown that the carbon dioxide carbon occurs in a carboxyl group. Fixation has been found to occur in the fermentation of sugars and other polyhydric alcohols by these bacteria, and in the decomposition of pyruvic and citric acids and in the formation of malic and fumaric acids from pyruvate by coliform bacteria. It has also been observed that carbon dioxide is assimilated by tissues of higher animals *in vitro*. It is of particular interest that fixation by heterotrophic bacteria may include the reduction of carbon dioxide by hydrogen in the presence of hydrogenase to form formic acid as noted earlier, and that a bacterium isolated by Barker, Ruben and Beck[105] produces acetic acid during the decomposition of purines which is synthesized from carbon dioxide, *i.e.*, radioactive carbon from the carbon dioxide was found in both methyl and carboxyl groups. The latter would seem to be a form intermediate between autotrophic and heterotrophic metabolic types of bacteria.

From present indications, therefore, it would appear that the heterotrophic assimilation of carbon dioxide is a general phenomenon. There is, as yet, however, no indication as to whether such assimilation is an essential and integral part of bacterial metabolic processes.

THE EFFECTS PRODUCED BY BACTERIAL GROWTH

It has been pointed out in an earlier chapter that bacterial growth may be regarded as a conversion of the constituents of the environment to bacterial protoplasm, a view which is an obvious oversimplification of the mass of highly complex and little understood reactions that constitute synthesis. This conversion is attended by a number of what might be termed "side reactions" which, in many cases, surpass in magnitude the synthesis of bacterial substance. It is to be expected, therefore, that a bacterial culture will contain, in addition to unaltered nutriment, first, materials arising as a result of "side reactions" which are, for the most part, degradations, and, second, bacterial protoplasm together with other substances synthesized by the organisms but not a part of the cell substance proper. The nature of the products so formed depends upon the kind of bacteria present, the kind of nutriments supplied and the conditions under which growth takes place.

The Products of Degradation.—Many of these have already been discussed under the heads of carbohydrate and amino acid decomposition.

[105] Barker, Ruben and Beck: Proc. Nat. Acad. Sci., 1940, *26*:477.

The presence of a fermentable sugar leads to the formation of organic acids and gases such as hydrogen, carbon dioxide and methane. The nature and proportions of these end products depend, of course, upon a number of factors, such as the acidity of the culture medium which may favor the formation of one organic acid and tend to suppress that of another; the degree of aeration which markedly influences the degree of oxidation or reduction of the accumulating compounds; the time of incubation, for accumulated compounds may be further decomposed upon continued incubation; and similar factors which exert a strong influence upon what might be considered as the primary determining factor, *i.e.*, the enzymatic equipment of the growing bacterium. Similarly, the hydrolysis of proteins by the proteolytic bacteria and the decomposition of amino acids by these and other organisms to amines, acids, saturated hydrocarbons, ammonia, carbon dioxide, hydrogen sulfide, hydrogen, and free nitrogen and oxides of nitrogen is determined by these same factors. A culture medium in which bacteria have grown may, and usually does, contain a complex mixture of these various metabolites.

In addition to these, certain other materials, which may also be considered in the category of metabolites, may be present. Chief among them are hydrogen peroxide and nitrite, which appear as the end products of coupled reactions in which organic compounds are oxidized and oxygen and nitrate are reduced. Although these substances cannot be reduced by all bacteria, their reduction is in no sense specific to any type of metabolic activity. With the exception of a few organisms such as the Shiga dysentery bacillus and certain streptococci, almost all bacteria produce hydrogen peroxide under favorable circumstances, but, since the majority of aerobic and facultatively anaerobic bacteria possess catalase, peroxide usually does not accumulate in the culture. It is probably not formed through direct coupling of oxygen reduction with substrate oxidation but possibly may arise via some mechanism such as the cytochrome-cytochrome oxidase system. Nitrate is reduced to nitrite by a number of bacteria, and to ammonia by some of the soil organisms, and is often included in simple synthetic media to permit anaerobic growth. The ability to reduce nitrate has assumed some importance as a differential character in the identification of some bacterial species. The reduction of sulfur compounds such as sulfite, thiosulfate, sulfate and the like to hydrogen sulfide is much less common than the reduction of nitrate. Although certain anaerobic bacteria found in muds reduce sulfate, the source of hydrogen sulfide in most bacterial cultures is the sulfhydryl group of cysteine.

The Products of Synthesis.—In addition to proteins, lipoids and carbohydrate in the usual amounts, many bacteria synthesize relatively large amounts of polysaccharide material which appears morphologically as the capsule surrounding the cell and which may confer an immunological specificity upon the bacterial species (p. 249). Enzymes are, of course, formed, and those that diffuse out of the cells are found in the culture medium. Two other substances have been of particular interest,

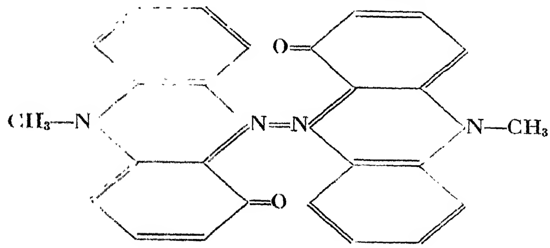
the pigments produced by the so-called chromogenic bacteria and the soluble toxins elaborated by some of the pathogenic organisms.

Bacterial Pigments.—Most bacteria do not form pigments, and masses of the cells, such as colonies on semisolid media, appear to be white or light gray. Some, however, give rise to colored compounds which may be brilliant in hue. Some pigments occur in solution, while others are found in the form of granules lying outside the cell. Practically all colors of the spectrum are represented: violet, indigo, blue (*Bacterium violaceum*, *B. janthinus*, *Pseudomonas pyocyaneus*), green (*Bacterium fluorescens*), yellow (*Staphylococcus aureus*, *Sarcina lutea*), orange (*Sarcina aurantiaca*), and red (*Bacterium prodigiosum*). The last-named, the so-called "miracle" bacillus, is sometimes the cause of the sudden appearance of blood-red spots on bread, rice, fish and other foods. A single bacterial species may contain more than one pigment; the purple sulfur bacteria, for example, contain lipochrome pigments in addition to bacteriochlorin.

Pigment production is probably one of the most unstable characters of bacteria even though it is often made use of as a differential character in many classification schemes. Some of the staphylococci may show pronounced yellow or golden yellow pigmentation upon isolation from an abscess, but the intensity of the color gradually fades with cultivation on laboratory media and may eventually disappear. Other organisms, such as *Bacterium violaceum*, may also lose chromogenic power through continued cultivation on artificial media, and cultivation of a chromogenic organism in the presence of dilute antiseptics may result in the complete suppression of pigment formation. Similarly, the incubation of *B. prodigiosum* at 37° C. results in cultures which entirely lack the brilliant red color characteristic of the organism. As a rule oxygen is indispensable to pigment production and most chromogenic species yield no trace of pigment when grown under anaerobic conditions. *Spirillum rubrum*, however, which grows well in the presence of oxygen, is said to form its red pigment only in oxygen-free media. In the case of some of the chromogens the presence of certain chemical compounds or elements in the medium is essential to, or greatly favors, pigment production. Thus phosphates and sulfates have been found necessary for the production of pyocyanin by *Ps. pyocyaneus*, and sodium tartrate has been shown to favor the production of pigment by *B. prodigiosum*. Carbohydrate media (potato, rice and wheat starch) often lead to a particularly brilliant chromogenesis.

The bacterial pigments are chemically of diverse nature. Many of the red and yellow pigments are insoluble in water, but soluble in alcohol, ether and chloroform, and appear to be lipochromes, a group of fatty pigments widely distributed throughout the plant and animal kingdoms; others, like the fluorescent pigment, are soluble in water, but not in ether or strong alcohol, and may be related to the anthracyanins. Of the bacterial pigments only the structure of pyocyanin is known; it has been found by Wrede[106] to be an entirely new type of dye and the first instance of a phenazine derivative occurring in nature. The empirical formula is $C_{26}H_{20}N_4O_2$ and the structure probably:

[106] Wrede: Ztschr. f. Hyg. u. Infektionskr., 1930, *111*:90.



The bacteriochlorin found in the purple and green sulfur bacteria appears to be closely related to chlorophyl *a*.

The relation of many of the bacterial pigments to the physiology of the organisms is uncertain. Bacteriochlorin is, of course, responsible for the photosynthetic activity of the purple and green sulfur bacteria. Cytochromes *a*, *b* and *c* are present in all bacteria except the obligate anaerobes, separately and in various combinations, and presumably function as a part of the respiratory mechanisms operative during aerobic growth. The presence of cytochrome, however, does not impart color to the bacteria. Other pigments such as pyocyanin may be reversibly oxidized and reduced and may conceivably function as hydrogen transport systems. The majority of bacterial pigments, however, fall into the group of lipochromes or carotinoids, and appear to be physiologically inert.

Toxins. The results of the parenteral inoculation of bacteria-free culture filtrates into experimental animals must be interpreted with considerable caution. A great variety of proteins, bacterial and otherwise, yield, on hydrolysis, split products of high molecular weight that are toxic, and the complex amines that may accumulate in the cultures of certain bacteria likewise give rise to marked symptoms, or even death, when injected intravenously. Some bacteria, however, have been shown to form what are called true toxins to distinguish them from the non-specific toxicity of the decomposing material present in the cultures of a variety of bacteria. These toxins diffuse out of the cells and may be found in the culture fluid and are, in consequence, also spoken of as "soluble toxins" or "exotoxins." They are apparently protein in nature, large molecules which are synthesized by the organisms capable of forming them, and they have many properties in common with enzymes. The ability to form toxins is one that is possessed by relatively few bacteria, the best known being the diphtheria and tetanus bacilli, *Clostridium botulinum* and the organism of symptomatic anthrax. These substances are, by a wide margin, the most potent poisons known. There is no explanation for this pronounced toxicity, for the soluble toxins do not differ obviously from other plant and animal proteins. Some of the pathogenic bacteria synthesize other substances termed hemolysins which bring about the dissolution of red blood cells. These substances also appear to be proteins. The cell substance of some bacteria, such as the cholera vibrio, some of the dysentery bacilli and others, is toxic upon injection presumably because of the presence of intracellular or endotoxins. The endotoxins differ in a number of

ways from the true toxins, and there is some question as to whether they represent toxic substances synthesized by the bacteria or whether the toxicity of the bacterial cells is a result of the decomposition of bacterial protein to toxic split products. The important subject of bacterial toxins is discussed at length elsewhere (Chapter 8).

The Physical Effects of Bacterial Growth. Both heat and light may be generated by bacterial growth. It has been pointed out earlier that bacteria use only a portion of the energy made available by the oxidation of organic compounds, the remainder being liberated as heat. When circumstances are such that the heat cannot escape as fast as it is generated, the temperature of the bacterial environment rises. The rise may be considerable; the heating of manure piles and damp hay in which bacterial decomposition is proceeding actively may bring them to temperatures as high as 70° to 75° C. Under such conditions the thermophilic bacteria become active and maintain these high temperatures. It has been suggested that spontaneous combustion may result from bacterial activity, but this seems improbable.

The phosphorescence sometimes observed upon decaying fish and meat is due to the growth of light-producing bacteria. The photogenic bacteria are found most commonly, though by no means exclusively, in sea water and upon the bodies of marine animals, and a considerable number of species have been described. Sodium chloride and magnesium chloride favor the growth of these phosphorescent bacteria, and one or the other of these salts is essential to the production of light. Luminescence is apparent only in the presence of oxygen; broth cultures from which dissolved oxygen has been exhausted by the respiratory activity of the organisms may be made to glow by shaking. Bioluminescence in certain crustacea, such as *Cypridina*, results from the oxidation of a substance, luciferin, by molecular oxygen in the presence of an enzyme, luciferase. Presumably a similar mechanism accounts for the production of light by the luminous bacteria, but neither luciferin nor luciferase has been isolated from these organisms.[107]

BACTERIAL ACTIVITY IN MIXED CULTURES (BACTERIAL ASSOCIATION[108])

Although the relatively precise knowledge which has made possible the development of bacteriology as a science has been gained through the study of bacteria in pure culture, it must not be forgotten that in nature a pure culture is the exception rather than the rule. Actively decomposing organic matter, for example, contains a great number of bacterial species, and a diseased animal may, and usually does, harbor a variety of microorganisms. The responsibility for a given phenomenon has, in many cases, been fixed upon but one kind of bacterium present in the naturally occurring mixture, the remainder having no apparent significance. The great

[107] Harvey: *Living Light.* Princeton University Press. Princeton. 1940. Also, Ann. Rev. Biochem., 1941, *10*:531.

[108] *Cf.* Holman: *Bacterial Association. Newer Knowledge of Bacteriology and Immunology.* University of Chicago Press. Chicago. 1928.

majority of infectious diseases are phenomena of this type, and it is possible, therefore, to speak of a given bacterium as the causal or etiologic agent of such a disease. Similarly, one microorganism may be shown to be responsible for one kind of fermentation while another takes place as the result of the activity of a quite different bacterium. Modern bacteriology clearly rests upon the generalization that may be made from these facts, the concept of specific microbial etiology. But is this the whole story? It may be asked, first, whether the microorganisms present in a mixed culture are passive with respect to one another or whether one species is affected, either adversely or favorably, by proximity to other actively metabolizing forms; and, second, whether or not a given phenomenon for which no single species can be shown to be responsible may result from the combined activities of a heterogeneous group of bacteria.

Beneficial Associations.—With regard to the first of these questions, bacteria have been found to exhibit the various degrees of relationship known to exist among the higher forms of life. Examples of *symbiosis*, a relation in which there is mutual benefit, are rare among the bacteria, but *metabiosis* or *commensalism*, in which one member benefits while the other is unaffected, is commonly observed. Perhaps the most obvious example is the growth of an obligate anaerobe in mixed culture with an aerobic organism. The latter uses up the oxygen in the immediate vicinity, thereby allowing the anaerobe to develop. Sporulating anaerobes are sometimes carried in culture with a non-spore-forming aerobe, for subsequent separation by heat is readily accomplished. In the breakdown of cellulose in the soil, the preliminary hydrolysis by the cellulose-decomposing bacteria yields glucose which may be utilized by a variety of organisms present which are unable to bring about the initial hydrolysis. Similarly, the hydrolysis of protein material by proteolytic bacteria liberates amino acids which may be further decomposed by non-proteolytic forms.

Bacterial Antagonisms.[109]—Bacterial antagonism or antibiosis is also of common occurrence. Fermentative and proteolytic types of bacteria generally do not prosper equally well in mixed culture. As pointed out previously, the bacterial proteases are for the most part tryptic in nature and work best in alkaline environments. The acid reaction resulting from the fermentation of carbohydrate is distinctly inhibitory to these organisms, a fact which has been the basis of attempts to replace the proteolytic flora of the large intestine with an aciduric flora consisting of organisms such as *Lactobacillus acidophilus*.[110] The formation of ptomaines or complex amines, whose absorption presumably leads to "autointoxication," by the proteolytic organisms is thereby suppressed or prevented. Numerous other examples of bacterial antagonism have been reported, many of them apparently somewhat more specific than acid inhibition. The well-known overgrowth of the diphtheria bacillus by *Staphylococcus aureus* is probably

[109] Waksman; *Microbial Antagonisms and Antibiotic Substances.* Commonwealth Fund. New York. 1945.

[110] *Cf.* Rettger and Cheplin: *The Intestinal Flora with Special Reference to the Implantation of "Bacillus acidophilus."* Yale University Press. New Haven. 1921. Also Kopeloff: "*Lactobacillus acidophilus.*" Williams & Wilkins Company. Baltimore. 1926.

the result of an antagonism. Other organisms are similarly antagonistic to the diphtheria bacillus; in mixed culture with Friedländer's pneumobacillus, for example, no diphtheria toxin is formed, and mixed infections with these two organisms usually run a mild course. Both *Bacterium coli* and *Bacillus mesentericus* are antagonistic to the diphtheria bacillus, but other organisms, such as the typhoid bacillus, may have no effect. *Bacillus mesentericus*, in turn, is without effect on a variety of other bacteria. Although antagonistic to the diphtheria bacillus, *Staphylococcus aureus* is itself markedly inhibited by the pneumococcus. Many similar examples of antagonism have been observed among the colon-typhoid group of bacteria, such as that between *Bacterium coli* and the dysentery bacilli. It is not unlikely that such relationships contribute in part to natural resistance to some kinds of infectious disease.

In general, non-spore-forming pathogenic bacteria are closely adapted parasites unable to maintain a saprophytic existence in nature, and persist for only a limited time apart from the host. This failure to survive is due in part to an unfavorable physical environment and in part to an inability to compete successfully with the free-living microorganisms. Furthermore, it has been found that the free-living forms are, in many instances, actively antagonistic to such pathogens as well as occasionally to one another. Thus the typhoid bacillus will survive two to four weeks in sterile water but less than a week in tap water, and dies out even more quickly in raw river or canal water. Similarly, the pathogens disappear relatively rapidly from soil when deposited there by elimination from the infected individual or earth burial of persons dying of infectious disease; there is, therefore, no hygienic objection to earth burial. The rate of disappearance of such bacteria is in part a function of the temperature, low temperatures tending to preserve them. It may be markedly accelerated by successive inoculations of a plot or sample of soil, a process which increases by selection the relative numbers of those microorganisms which feed upon or specifically destroy the inoculated bacteria.

Because of its rich and varied population the soil has proved a prolific source of antagonistic microorganisms even without the enrichment of repeated inoculation.[111] The method of isolation of antagonists for a particular bacterium is relatively simple. An agar plate is sufficiently heavily inoculated with the bacterium to produce a uniform film of growth and then streaked with soil. After incubation it will be found that the plate is covered with the film of growth of the inoculated bacteria together with colonies of microorganisms from the soil inoculum. Colonies of microorganisms antagonistic to the bacterium will be surrounded by a clear zone in the film of growth in which growth has been prevented. Such colonies may be picked and the microorganism subjected to whatever further study is desired. Microorganisms which are antagonistic to bacteria are sometimes other bacteria, but more often are higher fungi, molds and actinomycetes. (For the antagonism of higher plants to bacteria see p. 295.)

[111] Waksman and Woodruff: Jour. Bact., 1940, *40*:581.

[112] See Waksman: Bact. Rev., 1941, *5*:231; Amer. Jour. Pub. Health, 1944, *34*:358 and ref. 109.

The Antibiotic Substances.[112]—Investigation of antagonistic micro-organisms has shown that in many instances the antagonistic effect is due to the activity of a substance formed by the antagonist which is toxic for the affected bacterium. These substances have been termed *antibiotic substances* or *antibiotics* by Waksman. Such substances have been known for many years, the first, pyocyanin, having been isolated by extraction of "blue pus" in 1860 before the causative bacterium, *Pseudomonas pyocyaneus*, was discovered. Many others are now known. In general they are more effective on gram-positive bacteria, and differ from one another with respect to their relative toxicity for various species of bacteria.[113] Their chemical properties indicate that they are of diverse nature, including polypeptides, sulfur-containing compounds, lipids, pigments, quinones and organic bases. Some have been prepared in crystalline form and in a few cases the structure is known. These substances have become of greatly increased interest in recent years since it has been found that, while some are highly toxic to man, others are effective chemotherapeutic agents.

Antibiotic Substances from Bacteria.—PYOCYANIN AND PYOCYANASE.—The best known antibiotic substances of bacterial origin are those produced by *Pseudomonas pyocyaneus* and by aerobic sporulating bacteria of the genus *Bacillus*. *Ps. pyocyaneus* forms two substances having antibiotic activity, the chloroform-soluble phenazine compound *pyocyanin* (p. 128), and a substance known as *pyocyanase*, which appears to be a lipid, whose activity is associated with the presence of unsaturated fatty acids in the molecule. A third substance which also shows activity may be isolated by ether extraction; this is a yellow pigment, *hemipyocyanin*, and a derivative of pyocyanin. These substances are all relatively toxic to higher animals.[114]

TYROTHRICIN.—An alcohol-soluble, water-insoluble polypeptide having antibiotic activity, which he named tyrothricin, was isolated by Dubos[115] from a gram-positive aerobic sporulating bacterium, *Bacillus brevis*. Further investigation made possible its separation into two components, *gramicidin* and *tyrocidin*, of somewhat different properties though both contain "unnatural" amino acids of the *d* series.[116] Very similar, possibly identical, substances have been isolated from similar bacilli by a number of workers,[117] and bacteria such as *Bacillus subtilis*, *Bacillus mesentericus*, etc., also show activity but have not been studied in detail.

Antibiotic Substances from Actinomycetes.—A number of antibiotic substances have been isolated from actinomycetes (p. 599), especially by Waksman and his co-workers. The more important of these may be considered briefly. The first to be isolated were *actinomycin A* and *actinomycin B*, isolated from cultures of *Actinomyces antibioticus*.[118] The two substances, soluble in organic solvents, were both prepared in crystalline form, and

[113] Waksman and Woodruff: Jour. Bact., 1942, *44*:373.

[114] For recent studies see Schoental: Brit. Jour. Exp. Path., 1941, *22*:137; Stokes, Peck and Woodward; Proc. Soc. Exp. Biol. Med., 1942, *51*:126.

[115] Dubos: Jour. Exp. Med., 1939, *70*:1.

[116] Dubos, Hotchkiss and Coburn: Jour. Biol. Chem., 1942, *146*:421.

[117] See Stokes and Woodward: Jour. Bact., 1942, *43*:253.

[118] Waksman and Woodruff: Jour. Bact., 1941, *42*: 231; Waksman and Tischler: Jour. Biol. Chem., 1942, *142*:519.

the former found to be markedly bacteriostatic and weakly bactericidal, while the latter was chiefly bactericidal. Both are highly toxic to higher animals. Actinomycin A is a red pigment and appears to be a polycyclic nitrogen compound; it is usually referred to simply as actinomycin.

STREPTOTHRICIN AND STREPTOMYCIN.—A substance designated *streptothricin* was isolated from cultures of *Actinomyces lavendulae*.[119] This material is soluble in water and acid alcohol but not in ether, and appears to be an organic base. Its toxicity for higher animals is low. A very similar substance, *streptomycin*, was isolated from *Actinomyces griseus* and appears to be a closely related compound.[120]

Antibiotic Substances from Molds.—PENICILLIN. Of the antibiotic substances produced by molds, *penicillin*, found in cultures of *Penicillium notatum* and produced to a lesser extent by certain other penicillia such as *P. chrysogenum*, is by far the best known. This is largely because it is highly bacteriostatic and almost completely non-toxic for higher animals and has been found to be a highly effective chemotherapeutic agent.[121] It was first found by Fleming[122] in 1929 but its possibilities were not appreciated until the work of Oxford chemists[123] in 1940. Penicillin is readily soluble in water and is extracted from acidified aqueous solution with ether or amyl acetate, and may then be taken out of the organic solvent by shaking with dilute bicarbonate or barium hydroxide solution. It may be purified by chromatographic adsorption[124] and the sodium and barium salts are stable in the dry state. Some of its properties are described by Abraham, Chain and Holiday[125] but details of its nature have not as yet been made available in published form. Bacteria exposed to the action of penicillin become enlarged and swollen and fail to divide—direct evidence of its bacteriostatic action.[126] It is most active on gram-positive bacteria. The penicillins from different strains of the mold differ slightly in their action on various species of bacteria and in certain other minor respects. Three types or kinds have been recognized, and are known in this country as F, G and X, and in England as I, II and III.

Standardization.—The antibiotic activity of penicillin is assayed by inhibition of growth of standard strains of staphylococci. Growth may be measured by the turbidity of developing broth cultures, but the most commonly used method is that developed by the Oxford group and makes use of inhibition of growth on agar media. It consists of the inoculation of an agar plate so that a uniform film of growth will develop; small cylinders of glass or porcelain are set end down on the inoculated surface. One is filled with standard penicillin solution containing one *Oxford Unit* per

[119] Waksman and Woodruff: Proc. Soc. Exp. Biol. Med., 1942, *49*:207; Waksman: Jour. Bact., 1943, *46*:299.

[120] Schatz, Bugie and Waksman: Proc. Soc. Exp. Biol. Med., 1944, *55*:66.

[121] *Cf.* Florey and Florey: Lancet, 1943, *ii*:387; Committee on Chemotherapeutic Agents, National Research Council: Jour. Amer. Med. Assn., 1943, *122*:1217.

[122] Fleming: Brit. Jour. Exp. Path., 1929, *10*:226.

[123] Chain *et al.*: Lancet, 1940, *i*:226.

[124] See Abraham *et al.*: Lancet, 1941, *i*:177; Challinor and MacNaughtan: Jour. Path. Bact., 1943, *55*:441.

[125] Abraham, Chain and Holiday: Brit. Jour. Exp. Path., 1942, *23*:103, 115.

[126] Gardner: Nature, 1940, *146*:837.

cubic centimeter, and the others with varying dilutions of the unknown. After incubation there is a clear zone of growth inhibition around the cylinders, about 24 millimeters in diameter in the case of that containing the standard solution. The Oxford Unit is, then, defined[124] as "that amount of penicillin which when dissolved in 1 c.cm. of water gives the same inhibition (*i.e.*, area) as this standard." A tentative International Unit has now been adopted[126a] which is defined as the activity of 0.6 micrograms of the pure crystalline sodium salt of penicillin II or G, a quantity of which is available as a standard. This unit closely approximates the Oxford Unit. Present commercial preparations are as yet relatively crude and contain 150 to 160 Oxford Units per milligram in contrast to the crystalline preparations which contain 1650 Oxford Units per milligram. There is as yet no standard method of assay of the activity.[126b]

Substances very similar to penicillin have been found in other molds; *flavicidin*, produced by *Aspergillus flavus*, is one of these.[127]

NOTATIN.—A second antibiotic substance produced by *P. notatum* was described independently by British workers[128] as *notatin* and by American workers as *penatin*[129] and *penicillin B*.[130] It differs from penicillin in that it is insoluble in organic solvents and is active only in the presence of glucose. It appears to be a flavoprotein, producing hydrogen peroxide from glucose, and the peroxide is responsible for its antibiotic activity.

In addition to penicillin and notatin, a wide variety of antibiotic substances have been found to be produced by molds. Some of these are:

ASPERGILLIC ACID.—This substance is produced by *Aspergillus flavus*[131] and is active against both gram-positive and gram-negative bacteria. It is an amphoteric substance, soluble in alcohol, ether and acetone but not in petroleum ether, and its empirical formula is $C_{12}H_{20}O_2$. It is very toxic for higher animals.

PENICIDIN. This substance is also active against both gram-positive and gram-negative bacteria and is produced by a number of species of *Penicillium*.[132] It is extracted from culture fluid by ether at neutrality and precipitated from ether solution by the addition of petroleum ether as a yellow oil. Little is known of its nature.

PENICILLIC ACID. This substance is responsible for the antibiotic activity of some species of *Penicillium*, notably *P. puberulum*[133] and, in

[126a] See the recommendations of the International Conference on Penicillin: Science, 1945, *101*:42.

[126b] For studies on the assay see Foster *et al.*: Jour. Bact., 1943, *46*:187, 377; Rake and Jones: Proc. Soc. Exp. Biol. Med., 1943, *54*:189; Schmidt and Moyer: Jour. Bact., 1944, *47*:199; Sherwood, Falco and deBeer: Science, 1944, *99*:247; Joslyn: Science, 1944, *99*: 21. The method of assay used by the Food and Drug Administration is given by Hunter and Randall: Jour. Assn. Offic. Agr. Chem., 1944, *27*:430.

[127] McKee *et al.*: Jour. Bact., 1944, *47*:187.

[128] Coulthard *et al.*: Nature, 1942, *149*:634.

[129] Kocholaty: Jour. Bact., 1942, *44*:469; Arch. Biochem., 1943, 2:73.

[130] Roberts *et al.*: Jour. Biol. Chem., 1943, *147*:47.

[131] White and Hall: Jour. Bact., 1943, *45*:433; see also Jones, Rake and Hamre: *ibid.*, 1943, *45*:461.

[132] Atkinson: Australian Jour. Exp. Biol. & Med. Sci., 1942, *20*:287.

[133] Oxford, Raistrick and Smith: Chem. & Indust., 1942, *61*:22.

contrast to penicillin, is most effective against gram-negative bacteria. It is water-soluble and has been isolated in colorless crystalline form and found to have the empirical formula $C_8H_{15}O_4$. Two other metabolites of *P. puberulum*, *puberulic acid* and *puberulonic acid*, are also bacteriostatic. The former has been isolated as a colorless, crystalline dibasic acid with the empirical formula $C_8H_6O_6$, and the latter as bright yellow prisms with the formula $C_8H_4O_6$. Puberulic acid appears to be a quinonoid and puberulic acid the corresponding quinol.[134]

CLAVACIN (PATULIN).—This substance was isolated from *Aspergillus clavatus* and named clavacin,[135] and later from *Penicillium patulum* and called patulin;[136] the two were later found to be identical.[137] The name *tercinin* has been used as a synonym for patulin. It is most active against gram-negative bacteria. It is soluble in ether, chloroform, alcohol and water and has been shown to be anhydro-3-hydroxymethylene-tetrahydro-γ-pyrone-2-carboxylic acid. It shows greater bactericidal activity than most of the antibiotic substances. A substance isolated from *Penicillium claviforme*[138] and named *claviformin* has been found[139] to be identical with clavacin and patulin.

FUMIGACIN.—This substance has been found[135] in cultures of *Aspergillus fumigatus* and is active on gram-positive bacteria. It is soluble in alcohol and sparingly soluble in water and has been isolated in crystalline form. A substance designated *helvolic acid* has been isolated from the same species of *Aspergillus*[140] but its relationship to fumigacin is not clear.

CITRININ.—This substance is formed by *Penicillium citrinum* and acts on both gram-positive and gram-negative bacteria. It is soluble in water and in alcohol and appears to be an organic base.[141]

FUMIGATIN AND SPINULOSIN.—These substances were isolated from cultures of *Aspergillus fumigatus* and *Penicillium spinulosum* respectively.[142] Fumigatin has been found to be 3-hydroxy-4-methoxy-2,5-toluquinone and spinulosin is 6-hydroxy fumigatin. Fumigacin is the more active substance and is effective against *Staphylococcus aureus*, the anthrax bacillus and the cholera vibrio.

GLIOTOXIN.—This substance is produced by *Gliocladium fibriatum* and some species of *Trichoderma*[143] and has also been found admixed with fumigacin in cultures of *A. fumigatus*. It is soluble in chloroform and benzol alcohol and sparingly soluble in water. It has been isolated in crystalline form and found to contain both nitrogen and sulfur and possibly an

[134] Oxford, Raistrick and Smith: Chem. & Indust., 1942, *61*:485.

[135] Waksman, Horning and Spencer: Jour. Bact., 1943, *45*:233; see also Waksman: Science, 1944, *99*:220.

[136] Birkenshaw *et al.*: Lancet, 1943, *i*:625.

[137] Hooper *et al.*: Science, 1944, *99*:16.

[138] Chain, Florey and Jennings: Brit. Jour. Exp. Path., 1942, *23*:202.

[139] Chain *et al.*: Lancet, 1944, *ii*:112.

[140] Chain, Jennings and Williams: Brit. Jour. Exp. Path., 1943, *24*:108.

[141] *Cf.* Oxford: Chem. & Indust., 1942, *61*:48.

[142] Oxford and Raistrick: Chem. & Indust., 1942, *61*:128.

[143] Weindling and Emerson: Phytopath., 1936, *26*:1068; *ibid.*, 1937, *27*:1175; Johnson, Bruce and Dutcher: Jour. Amer. Chem. Soc., 1943, *65*:2005, Menzel *et al.*: Jour. Biol. Chem., 1944. *152*:419.

indol nucleus. It is effective on both gram-positive and gram-negative bacteria.

All of the antibiotic substances are much more active, *i.e.*, bacteriostatic in higher dilutions, than the sulfonamides, though less active than the dyes (p. 68). As pointed out earlier, they have been of considerable interest because of their possible utility as chemotherapeutic agents but the majority are too toxic for parenteral inoculation in higher animals, the outstanding exception being penicillin and possibly certain others such as streptothricin. The mechanism by which they inhibit bacterial growth is as yet completely unknown except in the case of notatin.

Bacterial Synergism.[144]—Association between bacterial species often affects the organisms in such a way that the ability of the individual species to decompose organic compounds may be modified or extended. This phenomenon, termed bacterial *synergism*, is sometimes qualified into "antagonistic" and "beneficent" synergism, but since the majority of the changes which indicate the combined work of two or more bacterial species cannot be construed as either favorable or unfavorable to the microorganisms, this distinction is often a difficult one to make. Perhaps the simplest examples of synergism are those in which the enzyme system of one organism supplements that of another. A mixed culture of a streptococcus which ferments lactose to acid but no gas, and one of the paratyphoid bacilli which do not ferment this sugar, in lactose broth produces an acid and gas fermentation. Similar results have been obtained with mixed cultures of non-sucrose-fermenting *Bacterium coli* and streptococci fermenting sucrose to acid. In such cases the colon-typhoid organisms further oxidize the split products resulting from the decomposition of the disaccharide to gas. Probably similar complementary action is the basis of increased yields of propionic acid in mixed cultures of propionic acid bacteria and lactic acid cocci over that obtained with the propionic acid organism alone. Other decompositions, however, are not so readily explained. Cellulose, for example, is often much more rapidly decomposed by bacteria in mixed culture than it is by component cellulose-decomposing organisms in pure culture.[145]

The course of decomposition may be qualitatively different in mixed culture from that in pure culture. It has been shown, for instance, that the acetone-butyl alcohol fermentation of *Clostridium pectinovorum* is transformed into a lactic acid fermentation by mixed culture with *Bacillus volutans*. A considerable number of pairs of organisms have been shown to produce gas from carbohydrates in mixed culture when neither alone is able to bring about this change,[146] and similarly, mixed cultures of *Clostridium chauvei* and *Bacillus paralactici* form butyl alcohol from glucose although neither alone could produce this change. This ability of mixed cultures of bacteria to bring about changes of which none of the component species is capable is of interest in connection with the concept of emergent evolution.

See the review by Burrows: *Synergistic Aspects of Bacterial Populations*. Biol. Symp., 1942, *8*:89

[145] Khouvine: Ann. Inst. Pasteur, 1923, *37*:711.

[146] Holman and Meekison: Jour. Inf. Dis., 1926, *39*:145.

One consequence of bacterial association has been of considerable interest in regard to mixed and secondary infections[147]—the alteration of the virulence of a pathogenic bacterium by association with other microorganisms. This alteration may be either a decrease or an increase in the pathogenic powers of the organism. The mild course of mixed infections of the diphtheria bacillus and Friedländer's bacillus has been referred to above, and in this case decrease in virulence is presumably due to inhibition of toxin formation. It is well known that inoculation with soil containing anthrax spores does not always produce the disease even though pure cultures isolated from the same soil are highly virulent. This apparent decrease in virulence is thought by some to have its explanation in the stimulation of the defense mechanisms of the animal by the extraneous bacteria present in the soil. On the other hand, virulence may often be increased by inoculation of a pathogenic organism in mixed culture. The mixture of *Bacterium prodigiosum*, a non-pathogenic form, with the bacillus of malignant edema increases the virulence of the latter so that sublethal doses for experimental animals become fatal. Similarly the virulence of the hemophilic bacteria (Chapter 24) is readily raised by inoculation of the experimental animal with mixed cultures. One of the most interesting examples of the effect of microbial association on virulence is that of swine influenza, in which it has been shown that the disease is produced through the combined action of a bacterium and a filterable virus, neither of which is able to produce the disease by itself. Phenomena such as this suggest that the etiology of some diseases may be a complex matter.

Knowledge of bacterial associations and the mechanisms which give rise to the observed results is in an unsatisfactory state. Experimental evidence is fragmentary, not in the sense of paucity of observation—the literature on the subject is a voluminous one—but because of a certain discontinuity which makes generalization difficult if not impossible. The interpretation of experimental results obtained with pure cultures is often difficult, and when mixed cultures of two or more different organisms are used, the difficulties are greatly multiplied. The loose use of terminology (some workers use symbiosis, metabiosis or commensalism, and synergism synonymously) is no doubt an inevitable consequence of lack of knowledge of the mechanisms involved. In spite of all this, however, the elucidation of many phenomena brought about by bacteria under natural conditions probably lies in the development of knowledge of the association of these organisms with one another.

It may be noted in passing that bacteria may live an associative existence with higher animals and plants. Symbiotic nitrogen fixation and the relation of the cellulose-decomposing bacteria to their herbivorous animal hosts are among the more obvious. Infectious disease of man is, of course, an example of antibiosis or antagonism.

[147] Bail: Ztschr. f. d. ges. Exper. Med., 1926, *50*:11.

CHAPTER 6

BACTERIAL HEREDITY AND VARIATION

Before the development of methods of isolating bacteria in pure culture many observers regarded these organisms as members of a markedly homogeneous group, possibly of but a single species, which showed a high degree of morphological variation. The interconversion of coccus, bacillary and spiral forms was considered to be of common occurrence and, in consequence, the observed morphological differences among the bacteria were held to be of no significance. This doctrine, designated as *pleomorphism*, reached its height with Nägeli[1] in 1877, who postulated but a single species to which all bacteria belonged. It should be noted that the term pleomorphism included not only morphological variation but equally facile alterations in virulence; the innocuous hay bacillus (*Bacillus subtilis*), for example, presumably could change suddenly into the highly virulent anthrax bacillus.

The development of pure culture techniques by Koch and others swung the pendulum to the other extreme, and the doctrine of *monomorphism* became predominant. It was held by Koch and other extremists, whose camp the great majority of bacteriologists soon joined, that a given bacterial species existed in one and only one form, and that aberrant forms were either evidence of contamination of the pure culture or were the so-called involution or degenerative forms which were dead or dying and therefore had no significance. This monomorphism was confined largely to bacterial form and, although abrupt changes such as the conversion of the hay bacillus to the anthrax bacillus were denied, alteration in the virulence of pathogenic bacteria was not regarded as inconsistent with an absolute constancy of form.

Despite the currency of this extreme monomorphism, evidence began to accumulate which indicated that, though most bacteria showed a remarkable constancy of form, morphological and physiological variation of these organisms is not uncommon. The last twenty or thirty years has seen the development of an extensive literature on this subject. Much of the evidence which has accumulated is of just sufficiently inconclusive character that widely different interpretations may be made. Contemporary workers fall into two groups in this respect: the conservatives, a group which includes the majority of workers, who still adhere to the doctrines of Koch and his school in a modified form and who accept the well established variations as observed facts whose full significance is as yet not apparent; and the radicals, who profess to see evidences of complex life cycles, conjugation, sexual reproduction and kindred phenomena in the reported

[1] Nägeli: *Die niederen Pilze in ihren Beziehungen zu den Infectionskrankheiten und der Gesundheitspflege.* Munich. 1877.

observations. It is not improbable that a sound interpretation lies somewhere between these views.

The biological significance of observed variations and their relation to similar phenomena occurring in the higher forms of life are difficult to assess. The difficulties stem in part from a strong tendency to regard bacteria as somehow "different" from other living organisms. This inherently sterile concept has arisen partly because of the absence of a morphologically discrete nucleus in the bacterial cell, partly because the characteristics of bacteria which vary are generally not directly comparable to the genetically determined characters which are studied in the higher organisms, and partly because the bacteria are subject to the influence of unique environmental factors.

The apparent lack of a nucleus precludes the use of cytological techniques so essential to the understanding of the genetics of the higher organisms. Although some workers[2] have postulated arrays of genes in the bacterial cell, the existence of such structures has not been demonstrated beyond doubt. As long as the genes of a bacterial cell exist only in terms of a hypothesis which many workers regard as doubtful they can hardly be made use of in the study of bacterial variation.

The differential characters which serve to distinguish bacteria from one another are, in general, of a greater degree of fineness than those which the biologist is accustomed to use. The bacteriologist is concerned with characters not as gross as the red and white eyes of *Drosophila* but which might be considered analogous to the individual enzyme systems operative in the formation of red and white eyes. When such characters have no counterpart among those known to be of fundamental significance to the higher organism, and most if not all of them as yet have not, it is extremely difficult to judge whether they are of deep-seated importance to the cell or whether they are biologically trivial. Loss of virulence by a pathogenic organism, while of great practical importance to its prospective host, may be of only minor significance to the bacterium. Analogies are, then, difficult to draw and are always of doubtful validity.

Not only are the environmental factors which affect the bacterial cell to some degree peculiar to unicellular organisms of minute dimensions, but the essential obscurity of the bacterial environment is, to no small degree, a consequence of the limits of human imagination. Some aspects of this bacterial environment are known. For example, the tremendously exaggerated effect of surface tension on objects as small as bacteria must profoundly influence bacterial form. The continuous molecular bombardment to which these organisms are subject and to which, because of their small size, they respond with the dancing motion of brownian movement, may be a factor which influences their structure and rigidity of form. The predominance of interfacial phenomena in the bacterial world of near-molecular dimensions undoubtedly contributes to its general character. There is evidence, for example, that hydrogen ion activity[3] and reducing

[2] *Cf.* Lindegren: Centralbl. f. Bakt., 1935, Abt. II, *92*:41; *ibid.*, 1935, Abt. II, *93*:113, 389.

[3] Peters: Trans. Faraday Soc., 1930, *26*:797.

intensities[4] may be quite different at interfaces from what the observer of gross phenomena assumes them to be. The environment to which these and undoubtedly other phenomena lend character is, clearly, an abstract one, and the interpretation of morphological or physiological changes taking place under such circumstances is, to say the least, difficult.

From these considerations it is easy to understand how bacteria can come to be regarded as "different" without real justification for such a belief. It cannot, then, be emphasized too strongly that, so far as is known, bacteria do not differ in any essential way from other living cells and any interpretation of variation or other phenomena must rest on a sound biological foundation.

Before discussing the types of bacterial variation which have been observed, two concepts fundamental to the general problem must be con-

Fig. 19.—The morphology of *Rhizobium*. Left, a smear from a pure culture on laboratory media; note the regularity of the "normal" bacillary morphology. Right, smear from a clover root nodule showing swollen, branched and coccoid forms, the so-called bacteroides. Fuchsin. × 1050.

sidered. One of the most important of these is that of the "normal" morphological or physiological state of a bacterium. The anthropocentric tendencies of the human mind stimulate the belief that the normal form and functions of a bacterium are those which are manifest in culture on the ordinary laboratory media. Certain evidence suggests, however, that the term normal is but a relative one. The symbiotic nitrogen-fixing organisms show a relatively constant "normal" morphology on the usual laboratory media, but in the root nodule they are found in a variety of bizarre forms designated as bacteroides. Which, then, may be considered to be the "normal" form, that observed in the laboratory or that shown by the organisms in what might be regarded as their natural habitat? The physiological differences between bacteria in pure culture and those in the mixed

[4] Korr: Jour. Cell. Comp. Physiol., 1938. *11*:233.

cultures occurring in nature have been discussed in the previous chapter. Clearly, then, the term normal, although an extremely useful one cannot be taken to have real meaning.

The second concept is that of bacteria in terms of populations. The existence of bacteria as population groups composed of enormous numbers of individuals places an unusually high premium on the survival value of any given individual character. A mutation occurring in an individual cell may well be swamped through sheer weight of numbers unless it has marked survival value. If some form of sexual conjugation takes place, as some workers suggest, a character without survival value would disappear even more rapidly. As will appear, the apparent flexibility in the response of bacteria to environmental conditions may have its roots in such population pressures.

Fluctuating Variations and Mutations.—Two kinds of bacterial variability are commonly recognized:

(1) Variations of the ordinary "fluctuating" types, which are distributed more or less systematically about a modal condition and may be grouped in a frequency curve or frequency polygon. As the term indicates, fluctuating variability swings to and fro, oscillating around an average type. The familiar deviations in human height have their counterpart in similar fluctuations in the size of individual cells in a bacterial culture. It has been emphasized by de Vries[5] that fluctuations remain fixed within certain limits, and that the accumulation of fluctuating variations can never give rise to a new quality in an organism or bring about the formation of a new species. It should be pointed out, however, that such fluctuating variations may be made to pile up by appropriate selection methods. Just as other organisms may be selected for a given character, so bacteria have been selected for size; the continued selection and culture of individual cells of *Bacterium coli* that were unusually long led to the establishing of strains that grew as long rods and showed no tendency to revert to the short bacillary form of the parent culture.[6] Such successful selection need not imply some form of sexual reproduction; different races of other unicellular organisms—protozoa—have been obtained by artificial selection without any sexual reproduction having occurred.

(2) "Discontinuous variations," "sports," or "mutations," which, on the other hand, are supposed to be perfectly definite changes which arise suddenly without the interposition of a series of intermediate forms, and having once appeared, are permanent and show no tendency to return to the mean of the parent form.

In general, bacteriologists will not hesitate to classify the variations with which they are most familiar as those of the fluctuating type. The number of these is indefinitely large, and, particularly in some groups, there is little doubt that they have been given too great classificatory value, as compared with precisely similar deviations in higher forms of life. At the same time these biologically trivial differences are often of no small practical importance, as in the study of pathogenicity.

[5] de Vries: *Mutationtheorie*. 2 v. Leipzig. 1901–03.

[6] Barber: Kansas Univ. Sci. Bull., 1907, *4*:1.

The question of bacterial mutation is more difficult. Sudden changes in bacterial cultures do occur; often these changes apparently overstep boundaries of "species" and "genera" formerly thought impassable. Some of these changes are reversible; others seem to result in the establishment of more or less "permanent" and "new" types of organisms. Many biologists stress two criteria in the differentiation of a mutation and a fluctuating variation; a mutation is supposed to occur suddenly and the resultant change in the organism to breed true, while a variation is slow and the development of new varieties through artificial selection requires many generations. Some bacterial changes would appear, then, to be mutations, but whether any of them is strictly comparable with the mutations among higher forms of life has yet to be determined.

Adaptations and Modifications.—Bacteria, like higher organisms, respond to changes in environment. This response may be manifested either as an alteration in the morphology or the physiological properties of the organisms. The production of bizarre forms through cultivation of some species of bacteria on media containing large amounts of salt is well known. External structures of the cell may be lost, regained or accentuated through suitable alterations in the bacterial environment. Capsules are generally more readily formed and considerably thicker in carbohydrate media than in solutions which do not contain fermentable sugars; flagella may be present when a motile organism is cultivated at one temperature but not at another, or may be suppressed by cultivation of the organisms in the presence of dilute antiseptics; spore-forming organisms such as the anthrax bacillus may become asporogenous when cultivated at higher than optimum temperatures or in the presence of dilute antiseptics, a change that may or may not be accompanied by a loss in virulence.

Attenuation.—Alterations in the virulence of pathogenic bacteria have been the subject of great interest because of the practical importance of this character. Loss of virulence, termed *attenuation*, is often brought about inadvertently by cultivation on artificial media and may be thrust upon an organism by cultivation under somewhat adverse environmental conditions. Cultivation at higher than optimum temperatures, alterations in the nutritive elements of or the inclusion of antisera in the medium may induce a loss of pathogenic power. Such reductions in virulence may be accompanied by morphological changes such as loss of capsule or alteration in the arrangement of the organisms with respect to one another, resulting in a change in colony appearance as in the so-called "rough" variants. These changes in morphology are apparently intimately associated with the virulence of an organism, but others, such as the loss to the anthrax bacillus of ability to form spores, are not the inevitable accompaniment of loss of virulence, since both asporogenous virulent forms and spore-forming avirulent forms of this organism have been found. Only careful investigation can show whether an association between virulence and morphology is indicative of an intimate relation between the two or whether it is purely fortuitous. The loss in virulence produced by growth in the presence of immune serum is often accompanied by an increased resistance on the part of the bacterium to the defense mechanisms of the host. Such changes may

occur in the animal body; typhoid bacilli isolated from chronic carriers are often rough, avirulent forms.

Still another type of change may be brought about in the pathogenic bacterium by its host. A microorganism highly virulent for one animal species and considerably less virulent for a second may be altered by continuous passage through the second animal host, the virulence for the first decreasing and that for the second increasing as the passage continues. The alteration of the virulence of the rabies virus by adapting it to rabbits and the conversion of the smallpox virus (variola) to cowpox (vaccinia) by animal passage are well-known examples of this type of change. The mechanisms operative in the loss of virulence are obscure, largely because the term itself does not refer to a single character of bacteria but rather to the combination of a series of characters, some of which are known.

Drug-Fastness.—An adaptation that is, at times, of therapeutic significance, is the development by a microorganism of an increased resistance to the effects of toxic substances. A microorganism having such increased resistance, particularly to drugs used in the treatment of disease, is said to be *drug-fast*. This adaptation may take place *in vivo* when an infection is treated by successive injections of a drug, or *in vitro* by cultivation in its presence. The development of drug-fastness is more frequently observed in the protozoan diseases, such as trypanosomiasis, than in the bacterial infections, largely because effective chemotherapy of bacterial infections has, in general, not been possible in the past, though it has been observed from time to time that *Treponema pallidum* may become resistant to arsenicals during the course of treatment of syphilis.

The development of a tolerance of or resistance to the effects of chemotherapeutic drugs has become of renewed interest with the widespread use of the sulfa drugs. It has been found by a number of workers that bacteria may become sulfa-fast by continued cultivation in the presence of the drug. Once established, the drug tolerance may persist for long periods of time. Sesler, Schmidt and Belden[7] observed that strains of pneumococci which acquired a high degree of resistance to sulfa drugs retained it indefinitely, while those which were only moderately resistant tended to lose their tolerance after removal from contact with the drug.

The nature of the modification has been of some interest. If the drug acts by interfering with some metabolic process (p. 72), it is not unreasonable to expect that sulfa-fast strains might differ physiologically from the parent strain, *i. e.*, metabolize by means of an alternative mechanism not susceptible to such interference. Such physiological differences have, in fact, been observed by MacLeod,[8] who has found that while sulfapyridine-fast strains of pneumococci dehydrogenate glucose at a rate equal to that of the parent strain, they cannot dehydrogenate 3-carbon compounds such as glycerol, lactate and pyruvate while the parent strain can. The acquisition of sulfa-fastness is also associated with a marked diminution in hydrogen peroxide formation. Since the action of the sulfonamides is antagonized by *p*-aminobenzoic acid, it has also been suggested that in some instances

[7] Sesler, Schmidt and Belden: Proc. Soc. Exp. Biol. Med., 1944, *56*:42.
[8] MacLeod: Proc. Soc. Exp. Biol. Med., 1939, *41*:215.

sulfa-fast strains of bacteria are resistant by virtue of an increased production of this or similar substances. In support of this Landy, Larkum, Oswald and Streightoff[9] have shown that sulfonamide resistance in *Staphylococcus aureus* is associated with increased synthesis of *p*-aminobenzoic acid. Similarly, McIlwain[10] has found that pantoyltaurine-resistant strains of *Corynebacterium diphtheriae* produce increased amounts of pantothenic acid which presumably antagonizes the analogue. In many cases, however, sulfa-fastness cannot be accounted for on this basis, and it is not unlikely that the mechanism of the adaptation differs from one bacterium to another and may be dependent upon the conditions under which the drug resistance was induced. However this may be, the resistance is specific, sulfa-fast bacteria being ordinarily susceptible to penicillin and so on. Harris and Kohn[11] have observed that even among the sulfa drugs there may be some specificity; the development of resistance of *Bacterium coli* to sulfonamide did not always parallel that to sulfathiazole.

A question of considerable practical importance is that of whether a bacterium can become sulfa-fast *in vivo* during therapy and be transmitted to other persons while remaining resistant. Thus it would be possible to develop a virulent, sulfa-fast strain by sulfa therapy that might become widely disseminated in the host population. Cases of infection with sulfa-fast strains of pneumococci have been found[12] and the development of sulfa-resistance during therapy has been observed.[13] In the latter instance the drug-fast strain was transmitted to a second person who developed pneumonia; after recovery both patients continued to carry the drug-fast strain. As yet, however, no widespread prevalence of infection with drug-fast strains has been observed, and according to Hamburger and his co-workers[14] the occurrence of drug-fast pneumococci is not quantitatively important in clinical practice. Few similar observations on streptococci have been reported.

With the general application of sulfa therapy in gonorrhea, the increasing proportion of sulfa-fast gonococci encountered has been striking. For example, Carpenter *et al.*[14a] have reported an increase in the incidence of such strains from 15 per cent to 59 per cent in a period of fifteen months; similar observations have been reported from England.[14b] It has been suggested that this marked increase may be due either to the development of sulfa-fastness *in vivo*, possibly in part through inadequate self-medication, or to an increased prevalence of sulfa-fast strains arising as a consequence of the elimination of susceptible strains by sulfa therapy. Landy and

[9] Landy, Larkum, Oswald and Streightoff: Science, 1943, *97*:265; see also Spink: Jour. Exp. Med., 1944, *79*:331.

[10] McIlwain: Brit. Jour. Exp. Path., 1943, *24*:203.

[11] Harris and Kohn: Jour. Immunol., 1943, *46*:189.

[12] Tillett, Cambier and Harris: Jour. Clin. Invest., 1943, *22*:249.

[13] Frisch, Price and Myers: Ann. Int. Med., 1943, *18*:271.

[14] Hamburger, Schmidt, Sesler, Ruegsegger and Grupen: Jour. Inf. Dis., 1943, *73*:12.

[14a] Carpenter, Ackerman, Winchester and Whittle: Amer. Jour. Pub. Health, 1944, *34*:250.

[14b] *Cf.* Alexander: Brit. Med. Jour., 1944, *ii*:162. Taylor and Barlow: *ibid.*, 223; Johnson: *ibid.*, 321.

Gerstung[14c] have found that at least some sulfa-fast gonococci, like sulfa fast staphylococci, produce increased amounts of *p*-aminobenzoic acid. Resistance to sulfa therapy may, however, be more than infection with a sulfa-fast strain; Boroff[14d] has shown, for instance, a correlation between resistance to therapy and the antisulfonamide activity of the patient's serum.

Physiological Variation.—Variation in the more obvious physiological properties of bacteria, such as the ability to decompose proteins, ferment various sugars and produce pigment, has been known for a long time. This physiological variation may be considered, for practical purposes, as falling into two categories.

Mutation-Like Variation.—One of these, a mutation-like change, was described in 1907 by Massini,[15] who isolated a non-lactose-fermenting strain of *Bacterium coli* which, when cultivated on lactose agar containing an indicator which turned red in the presence of acid, gave rise to white colonies. On continued incubation red papillae appeared on the white colonies, indicating that some of the organisms were decomposing the sugar to acids. Subcultures from the red papillae bred true and showed no tendency to revert to the non-lactose-fermenting form, while subcultures from the white portions of the colonies gave rise to white colonies upon which red papillae appeared as before. A similar mutation-like change with respect to citrate utilization by coliform bacteria has been observed by Parr and Simpson.[16] Extensive experiments of this general type by other workers have indicated that this kind of variability is not uncommon among members of the colon-typhoid-dysentery group of organisms. A number of these organisms will, when cultivated on media containing a sugar they are unable to ferment, respond with the formation of such papillae, the tendency toward reversion to the parent type varying from one organism to another.

Adaptive Enzymes.[17]—The second type of physiological variation is that in which the ability of an organism to decompose a particular substrate is markedly enhanced by cultivation in the presence of that substrate. Although known for many years this particular type of bacterial variation has been the subject of renewed interest recently under the title of *enzyme adaptation;* the enzymes which may be formed under the influence of the presence of substrate have been called "adaptive" enzymes as contrasted with those which are formed whether the substrate is present or not, the "constitutive" enzymes.[18] Washed suspensions of the colon bacillus, for example, bring about an equally rapid decomposition of glucose regardless of whether the medium upon which the organisms were grown contained this sugar. On the other hand, the conversion of tryptophane to indol by washed suspensions of this same organism is much more rapid if the bacteria are grown on a medium containing tryptophane than if the medium did

[14c] Landy and Gerstung: Jour. Bact., 1944, *47*:448.
[14d] Boroff: Bull. U. S. Army Med. Dept., 1944, *78*:111.
[15] Massini: Arch. Hyg., 1907, *61*:250.
[16] Parr and Simpson: Jour. Bact., 1940, *40*:467.
[17] For recent reviews see Dubos: Bact. Rev., 1940, *4*:1; Gale: Bact. Rev., 1943, *7*:139
[18] *Cf.* Karström: Ergeb. Enzymforschung, 1938, *7*:350.

not contain this amino acid. Many of the bacterial proteases are adaptive enzymes, for only small amounts of the enzyme are formed in the absence of protein, but filtrates from cultures in protein-containing media are actively proteolytic. In cases such as these the adaptation is relatively rapid; a single transfer on the appropriate medium suffices to bring about the formation of the enzyme in appreciable amounts. In others, however, the adaptation may be a slower process and a series of transfers on a substrate-containing medium may be necessary before the bacterial culture develops the ability to form enyzme in detectable or maximum amounts. An essential part of this adaptation appears to be cell multiplication; it has been specifically shown in one case, that of the galactozymase of *Bacterium coli*,[19] that the amount of enzyme formed was proportional to the number of new cells. There are, however, two cases on record in which enzyme was formed by washed suspensions of bacteria in the presence of the substrate and under circumstances such that cell division did not occur

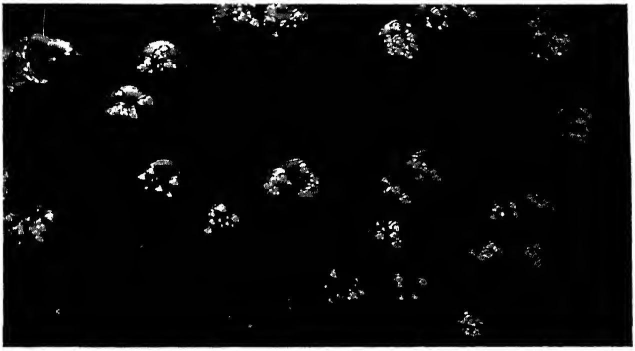


Fig. 20.—Colonies of *Bact. coli mutabile* on lactose agar. Note the lactose-fermenting papillae of the variant appearing on the non-lactose-fermenting colonies (Parr).

to an appreciable extent.[20] In these experiments the washed bacteria initiated the decomposition of the substrate only after an initial latent period during which the enzyme was presumably formed; plate counts and total counts indicated no increase in numbers of cells. In the vast majority of cases, however, multiplication is an essential part of the adaptation process.

Perhaps analogous to the phenomenon of enzyme adaptation is the conversion of nutritionally fastidious bacteria to a less exacting physiological state. Some strains of *Staphylococcus aureus*, for example, although initially requiring a number of amino acids, have been adapted to grow in the presence of an ammonium salt as the only source of nitrogen.[21] The implication is, of course, that the ability to synthesize the amino acids

[19] Stephenson and Gale: Biochem. Jour., 1937, *31*:1311.

[20] The formic hydrogenlyase of *Bact. coli* (Stephenson and Stickland: Biochem. Jour., 1933, *27*:1528) and the galactozymase of *Saccharomyces cerevisiae*, a yeast (Stephenson and Yudkin: Biochem. Jour., 1936, *30*:506).

[21] Gladstone: Brit. Jour. Exp. Path., 1937, *18*:322.

has been acquired by the bacteria. Whether this is generally true is open to question.[22] Similar but less definite nutritional changes are well known; many of the pathogenic bacteria are difficult to cultivate when first isolated but may grow more readily on laboratory media with continued transfer, a change which is not infrequently accompanied by a diminution in virulence as noted above.

These bacterial variations, both morphological and physiological, are clearly the result of the influence of the environment upon the organisms and may be regarded as adaptations to such external influences. It is already apparent that the great majority of these variations are in the nature of losses to the organisms of one or more qualities which may or may not be associated with one another. Such losses are not necessarily permanent, for some qualities so lost may be regained by cultivation of the organisms under suitable conditions. Whether the loss of some quality, such, for example, as the ability to ferment a given sugar or to invade the tissues of a host, and the subsequent regaining of that quality, may be regarded as analogous to the loss of an anatomical structure by a higher animal is open to serious question. It is, of course, extremely difficult to demonstrate the complete loss of a quality by a bacterial culture; the quality may still be present but to a degree too small to be detected. It cannot be said, then, that bacteria are capable of regaining lost characters in the generally accepted sense of the term.

The question of the nature of the environmental effect which results in the observed bacterial variations is of considerable general biological importance. In some cases the effect is obviously the result of a mild toxic influence. The adverse effects of higher than optimum temperatures, of high salt concentrations, of reduced surface tension and the like, which result in the appearance of "abnormal," bizarre forms, is, in all probability, traceable to such a toxicity, and the so-called "involution" forms so frequently observed in old cultures may plausibly be charged to the toxic effects of accumulated waste products. Morphological alterations so produced are for the most part but transitory, for the organisms revert to a "normal" form immediately upon transfer to ordinary media. Some of the changes so induced, however, may be of permanent or semipermanent character. The loss of capsules or flagella or the ability to form spores may be regained either relatively slowly, *i. e.*, by repeated transfer, or not at all.

The physiological variations produced by cultivation of the organisms under conditions favorable to "normal" growth and designed to bring about or make apparent the desired variation may be interpreted as the result of selection either of a fluctuating variation or a mutation. The mechanisms underlying the two types of change are, of course, profoundly different, the first presuming the existence of the quality under consideration and the second postulating that it arises *de novo*. Unfortunately the practical necessity for the study of bacteria as large populations has made the differentiation of these two types of change impossible. It is obvious that if the environment of a developing bacterial population is altered by

[22] Burrows: Jour. Inf. Dis., 1939, *64*:145; *ibid.*, 1939, *65*:134.

the inclusion, for instance, of a fermentable sugar, the ability to oxidize that sugar is thereby given a marked survival value and the character of the population as a whole may be altered accordingly. Such an experiment cannot, by its very nature, indicate whether the organisms inoculated into such an environment already possessed the ability to ferment that sugar, albeit to a quantitatively insignificant extent, or whether the ability to ferment the sugar arose as a chance mutation which, because of the survival value given it by the environment, remained to alter the physiological character of the population.

Further study has not as yet provided a solution to the purely technical problem of the obscuring effect of the bacterial population. For example, Massini concluded that the lactose-fermenting variants of his non-lactose-fermenting strain of *Bacterium coli* arose as a result of a mutation in the genetic sense because they appeared suddenly and bred true, and, in consequence he named the parent strain *Bacterium coli mutabile*. More recent work[23] has indicated that a relatively constant proportion, about one in 100,000, of the cells of *mutabile* strains are able to ferment lactose. It may be concluded either that (a) such strains are genetically unstable, the probability of the lactose-fermenting mutant arising being 0.00001, or (b) the ability to ferment lactose is possessed to a greater or lesser degree by all the cells but only in one in 100,000 (representing the tail of a frequency distribution) is the ability sufficiently marked to allow detection by the experimental procedure used.[24] Interesting observations bearing on this point have been reported by Bunting[25] who studied the distribution of color variants arising in aging cultures of the red pigmented organism, *Bacterium prodigiosum*. Consideration of other examples of physiological variation leads to essentially the same result, the inability to distinguish the basic mechanism through the haze of the bacterial population. It may be said, then, that mutation, using the term in the usual biological sense, may or may not occur in the bacteria, for as yet there is no conclusive evidence.[26] In the case of the fungus *Neurospora crassa*, however, it has been shown by Tatum and Beadle[27] that x-ray irradiation produced mutants which required *p*-aminobenzoic acid, *i. e.*, were presumably unable to synthesize it, though the parent strain did not require it; the essential step in the synthesis apparently was controlled by a single gene.

Bacterial Life Cycles.—The question of the existence of bacterial life cycles analogous, perhaps, to those long known among the higher fungi, is one which has been answered only inconclusively. Certain forms of cyclical development reflected in a succession of morphological types have been well established.[28] Among the simplest of these are the spore-vegetative cell succession of the sporulating bacteria and the successive zoogleal and monad or swarmer stages of *Nitrobacter* species. The root nodule bacteria,

[23] Lewis: Jour. Bact., 1934, *28*:519.

[24] The utilization of lactose as a sole source of carbon in a synthetic medium.

[25] Bunting: Jour. Bact., 1940, *40*:57, 69: *ibid.*, 1942, *43*:585, 593.

[26] For a somewhat different discussion and theoretical consideration of the entire problem see Yudkin: Biol. Rev., 1938, *13*:93.

[27] Tatum and Beadle: Proc. Nat. Acad. Sci., 1942, *28*:234.

[28] The cytomorphosis theory of Henrici has been discussed elsewhere (p. 53).

the *Rhizobia*, exhibit a somewhat more complex series of forms which have been divided into five groups.[29]

(1) a non-motile, coccoid, pre-swarmer form,
(2) a larger non-motile coccus having approximately double the diameter of the pre-swarmer form,
(3) a motile swarmer stage in which the cell acquires flagella and assumes an ellipsoidal form,
(4) a less actively motile rod form arising as a result of further elongation of the swarmer, and
(5) a vacuolated stage in which the chromatin divides into a number of bands which escape from the rod as the coccoid pre-swarmer.

This interpretation has, however, been criticized by Lewis.[30]

Similar successions of morphological types have been postulated for many of the well-known bacteria such as the staphylococci, streptococci and the gram-negative intestinal organisms of the colon-typhoid-dysentery group. With the exception of the cases noted above, the evidence for the existence of such life cycles is exceedingly tenuous for two reasons. Much of it is open to serious technical criticism,[31] and in all cases there are equally plausible alternative explanations. Aside, then, from the point of technical criticism, differences in opinion are largely a matter of interpretation and, in the absence of conclusive experimental evidence, which has as yet not been forthcoming, are likely to remain so for the time being.

The experimental evidence upon which the belief in bacterial life cycles is based consists of observations of aberrant, "abnormal" forms occurring in pure cultures. It is postulated that the so-called "normal" bacterial form is but one morphological stage of many in which a bacterium may exist but in which it is temporarily fixed by the constant environment of the laboratory medium. The appearance of aberrant forms is, then, evidence of a tendency on the part of the organism to assume the forms characterizing other stages of the life cycle, and such forms should not be termed involution or degenerative forms. The aberrant cells in turn break down to liberate minute viable organisms either directly or via a symplastic stage. The minute forms, designated as gonidia, gametes, microgametes and the like, may give rise to the "normal" vegetative cell, either directly or after a process of conjugation or sexual union.

In any attempt to weigh the evidence in favor of bacterial life cycles two points must be borne in mind. In the first place, the aberrant forms which constitute an essential part of the life cycle occur only in the death phase of a bacterial culture, never during the phases of active growth; and, in the second, many of the postulated forms are too small to permit resolution with visible light. Alternative explanations of aberrant morphology are many. For example, no small degree of structural rigidity is necessary to maintain a bacillary form in the face of the forces of surface tension, and any crumbling of that structure will of necessity result in

[29] Bewley and Hutchinson: Jour. Agr. Sci., 1920, *10*:144.
[30] Lewis: Jour. Bact., 1938, *35*:573.
[31] For example, see Holman and Carson: Jour. Inf. Dis., 1935, *56*:165; see also Lamanna: Jour. Bact., 1944, *47*:327.

misshapen cells. The ballooning of coccus forms followed by disruption with the liberation of granular material has often been regarded as evidence of life cycle phenomena. Suppose the cell dies, autolysis follows with the breakdown of cell constituents to smaller molecules, the increase in the number of molecules in solution raises the osmotic pressure within the cell and the cell imbibes water with resultant distention and eventual disruption. Granular material may be volutin and similar granular substances commonly observed in bacterial cells.

It is perhaps significant that cultural studies of the aberrant forms have indicated that in many cases these forms are dead and incapable of multiplication and may, therefore, be properly regarded as degenerative or involution forms. Other studies utilizing the micro motion picture technique for the continuous observation of the development of bacteria from a single cell have yielded no evidence of a bacterial life cycle.[32] It may be

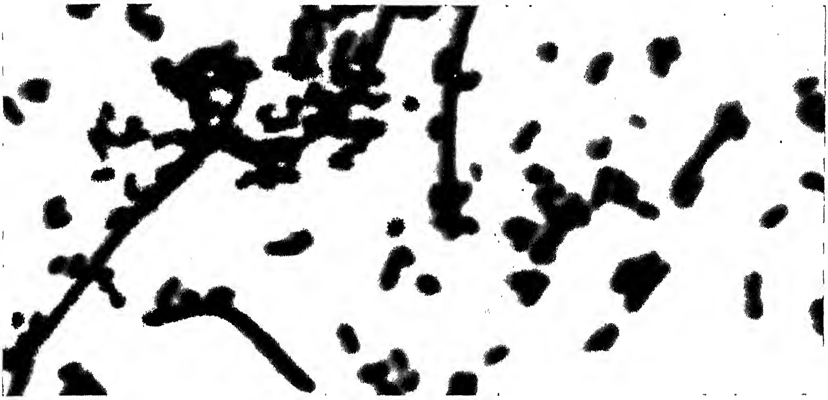


Fig. 21.—Involution forms of the typhoid bacillus; fuchsin stain. Note the filamentous forms and elongated rods mixed with the typical forms. What appear to be buds on the filament are probably adjacent cells. The poor resolution at these high magnifications is apparent. × 3500.

concluded, then, that bacterial life cycles, hybrids, conjugation and sexuality should be regarded as possibilities but not as proved facts.

Filterable Forms of Bacteria.[33]—Perhaps associated with bacterial life cycles are the so-called filterable forms of these organisms. The visible, readily cultivable forms of bacteria such as are present in the ordinary "pure culture" do not usually pass through the finer-pored porcelain and infusorial earth filters. That there are particular conditions, however, that favor the transmissibility of certain bacteria through these filters has become increasingly evident. Aside from the technical difficulties that beset all filtration experiments there are serious divergences of interpretation. Many observers have reported that familiar microorganisms such as the tubercle bacillus and the typhoid bacillus can sometimes pass through

[32] Wyckoff: Jour. Exp. Med., 1934, *59*:381.

[33] Hadley, Delves and Klimak: Jour. Inf. Dis., 1931, *48*:1.

well-constructed filters and this they interpret as signifying the existence of a filterable stage in the life history of these bacteria. It is even alleged by some that the filterable viruses of smallpox, poliomyelitis and other diseases represent a filterable phase in the life cycle of visible microorganisms, streptococci or what not, a view that has been urged especially by Rosenow[34] in this country and Enderlein[35] in Europe. This view is by no means generally accepted.

On the other hand, it is maintained that the so-called "filterable" forms of the typhoid bacillus, for example, are merely cells dwarfed by inadequate nutrition or are viable cell particles so small as to pass through filters but with capacity of renewed growth when again placed in favorable surroundings. It may be urged that the ability of cell fragments to regenerate is no new thing in biology, and it is quite as plausible to regard minute filterable forms of cocci or tubercle bacilli as portions of fragmented cells as to look on them as representing a significant filterable phase in their life histories.

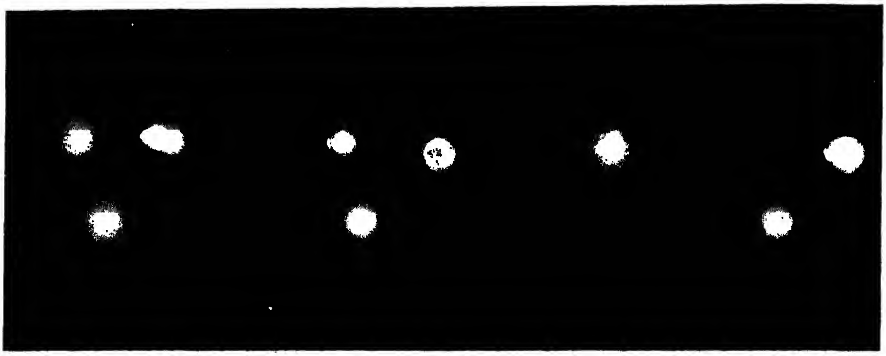


Fig. 22.—Smooth and rough colonies of *Bact. typhosum;* × 3. Culture on nutrient agar inoculated from an old broth culture.

Bacterial Dissociation.—A remarkable type of bacterial variation, whose most obvious outward manifestation is a change in the type of colony formed on semisolid media, was observed by Baerthlein in Germany (1918), Arkwright in England (1921) and de Kruif in the United States (1921). The phenomenon was termed by de Kruif bacterial or microbic dissociation, a term which has, in spite of certain undesirable features, been universally adopted.

The ordinary laboratory culture, particularly old broth cultures, when plated out on an agar medium develops into two kinds of colonies; one, smooth (S), round, convex and shining; the other, rough (R), irregular, flattened and wrinkled. In addition to these obviously different extreme colony types (Fig. 22), all degrees of intermediate (SR and RS) types may usually be found. The transformation of the S form, usually regarded as the "normal," into the R type is, as indicated by the presence of inter-

[34] For example, see Rosenow: Amer. Jour. Clin. Path., 1944, *14:*150.
[35] Enderlein: Arch. Entwicklungsgesch. Bakt., 1940, *1:*252.

mediate types, probably a gradual process. The smooth form of the great majority of bacteria, while breeding true for a number of test tube generations, shows a constant tendency to change to the rough type; one of the consequences of this tendency is that most stock laboratory cultures contain a large proportion of roughs, and sometimes such cultures are completely lacking in smooth forms.

The transformation of S to R, though occurring in ordinary cultures, may be hastened by a number of procedures, such as the inclusion of lithium chloride or anti-S immune serum in the medium in which the organisms are cultured. Smooth cultures lysed by bacteriophage, a filterable virus parasitic on bacteria (Chapter 38), give rise when incubated to a secondary growth which consists entirely of R forms. The change from R to S is considerably more difficult, and the rough forms show little tendency to revert under ordinary cultural conditions. The reversal may, however, be accomplished in most cases, the best test tube method being the cultivation of the R form in the presence of anti-R immune serum. Reversion of the R type may also be brought about by animal passage.

The bacteria which make up the smooth and rough colonies differ from one another in a number of characteristics, some of which are usually regarded as of deep-seated biological significance. These are most conveniently considered in outline form:

Smooth (S) Type	Rough (R) Type
(1) Broth cultures uniformly turbid	(1) Sedimental deposit in broth cultures; supernatant fluid clear
(2) Suspensions in salt solution (0.85 per cent NaCl) stable and remain cloudy	(2) Suspensions in salt solution clump spontaneously and settle out
(3) Flagellated species usually motile	(3) Motility reduced or absent
(4) Capsulated species show capsules	(4) Capsules absent
(5) Somatic and flagellar or type specific antigens present; flocculent agglutination	(5) Only somatic antigens present; agglutination granular in type
(6) Pathogenic species generally virulent	(6) Virulence greatly reduced or absent
(7) Biochemically active	(7) Biochemical activity reduced
(8) "Normal" morphology	(8) Tendency toward abnormal forms

The distinctions between the S and R types as outlined here represent a generalization to which there are a number of exceptions. For example, what is usually regarded as the "normal" form of the anthrax bacillus is the rough form and in this case the organism is virulent in the rough form but avirulent in the smooth form. Similarly, the "normal" virulent form of the hemolytic streptococci is finely granular rather than smooth, and the avirulent form which arises from it has lost its type specific antigen but is smoother than the virulent form. In spite of these and other exceptions the differences noted above hold true in general.

Closer inquiry into the differences between S and R bacterial types indicates that the relation between colony form and certain other characters is not in the nature of a casual correlation but rather the result of an intimate association. The association between capsules and virulence has been pointed out previously, as has been the rôle of the capsule in giving the colony of capsulated bacteria a slimy, viscous consistency. It is not

unreasonable to expect, then, that a bacterium which lacks a capsule is also avirulent, or relatively so, and that its colonies will be dry and possibly wrinkled in appearance. The loss of a capsule likewise alters the nature of the bacterial surface; the absence of carbohydrate material allows the lipoids present in the cell membrane to determine its character; in consequence, it becomes hydrophobic rather than hydrophilic and the organisms tend to clump and settle out of salt solution or the nutrient broth of a culture. The granular consistency of many of the rough colony types is accounted for, in part, by post-fission movements of the cells. The smooth forms of some of the intestinal bacilli separate after fission and slip to lie side by side with one another. The cells of the rough forms, on the other hand, tend to remain attached after fission with the formation of chains which bend at sharp angles here and there. The development of bizarre shapes by some of the rough forms may possibly be a result of the absence of capsular material and change in the nature of the cell surface. The nature of the association between roughness and reduced biochemical activity is unknown.

The relation between the S–R variation and the fluctuating variations and mutations discussed earlier is unknown. Clearly the change is not fluctuating in character and its reversibility would apparently rule out mutation. The dissociation is, of course, influenced by the environment, and in all probability some of the alterations considered as adaptations are closely connected with the occurrence of smooth and rough forms. Possibly other factors, such as antagonisms between the two types, play a part in the dissociative change, but to date evidence is lacking.

The G Colony.—A third type of colony, the so-called G type, although apparently seen by earlier observers in 1910 and 1911, was brought to general notice by Hadley and his co-workers.[33] These colonies are very small, 0.05 mm. or less in diameter, and appear to be made up of small cells of diverse morphology. Since these colonies are the first to appear in cultures of filtered bacterial cultures, they are assumed by some workers to represent a virus-like phase in the life history of the micro-organism which has been referred to above. Other workers, however, regard them as slow-growing variants at a low level of vigor and, for the present, their significance must be regarded as uncertain. Reversion of the G type to the parent form takes place gradually with apparently full resumption of the characteristic ancestral morphological, physiological and serological properties.

Immunological Variation.—The loss of capsules in the conversion of S to R forms is reflected, not only in the morphology and virulence of the organism, but also in its immunological character.[36] The capsular material, usually polysaccharide in nature, confers a type specificity upon the organism, while the cell body proper contains antigens, designated as somatic, which confer an immunological relation upon closely related forms having similar or identical somatic antigens but different type specific antigens. The change from S to R is, then, ordinarily accompanied by a loss of immunological specificity. The pneumococcus types, for example, differ

[36] The antigenic composition of bacteria is discussed in more detail on pp. 252, 382.

from one another by virtue of type specific capsular material and in the rough form lose their type specificity and become immunologically identical (p. 340).

Another type of colony variation or dissociation connected with the presence or absence of flagella was originally observed in cultures of *Proteus vulgaris*.[37] The ordinary type of *Proteus* colony is irregular and tends to spread in a thin film over the surface of an agar medium because of the pronounced motility of these organisms, but occasionally compact, discrete colonies are observed. The spreading type has been designated by the letter H (German *Hauch* = film) and the discrete form by the letter O (German *ohne Hauch* = without film). The distinction between H and O forms has come to have considerable significance because of the difference in the antigens present in the flagella and those present in the cell body,[38] the H or flagellar antigens conferring, like the capsular polysaccharide of some organisms, a type specificity upon the bacterium. The O antigens, though often referred to as somatic antigens, are by no means strictly analogous to the somatic antigens of the rough forms of bacteria, as will appear.

The nature of the H–O variation would appear to be different from that of the S–R variation in that the former takes place somewhat more readily and does not have a semipermanent character. The formation of flagellar antigen, for example, may be suppressed by cultivation of the bacteria on phenol agar (0.1 per cent phenol), a suppression which is immediate but not permanent, for transfer to nutrient agar results in the prompt reappearance of the H antigens. Furthermore, the H antigens of the *Salmonellas* have been found to be of two kinds, specific and non-specific (the non-specific having even broader group affiliations than the O antigens). Bacterial species having both specific and non-specific H antigens are designated as diphasic. When plated out they give rise to approximately equal numbers of morphologically identical but immunologically distinct types, one having specific H antigens and the other non-specific H antigens. This variation would appear to be of a fluctuating type, since the two types of colonies breed true for but a brief time, both reverting after one or two transfers to mixtures of the two types. (See also p. 382.)

The relations between the H–O and the S–R types of variation are not as yet clearly understood. In general, as has been stated, the R types are non-motile, but motile (MR) strains sometimes occur, and, conversely, non-motile S types (NMS) are observed. The conversion of some of the *Salmonella* species from the S to the R form is accompanied not by a loss of flagellar antigens but by an alteration in the immunological specificity of the somatic antigens,[39] possibly attributable to the loss of polysaccharide.

The Transmutation of Immunologic Types.—Perhaps one of the most important contributions of recent years to the problems of immunologic variation among the bacteria is the alteration of the pneumococcus

[37] Weil and Felix: Wien. klin. Wchnschr., 1917, *30:*1509.
[38] Smith and Reagh: Jour. Med. Res., 1903, *10:*89.
[39] *Cf.* White: Medical Research Council (British). *A System of Bacteriology*. Vol. 4, pp. 86–158.

types. It was shown by Griffith[40] that if a Type I pneumococcus, for example, is made to lose its type specific polysaccharide (capsule) by conversion to the R form, then injected into an animal together with a suspension of heat-killed smooth Type II pneumococci, the living organism cultivable from the animal is a Type II. It was later found[41] that this transmutation of type could be brought about in the test tube by cultivation of the R form in the presence of anti-R immune serum and heat-killed smooth pneumococci of another type. This remarkable change is well established, and errors of experimental technique have been eliminated.[42] Furthermore, Avery, MacLeod and McCarty[43] have isolated a substance from Type III pneumococci, consisting principally, if not solely, of a highly polymerized viscous form of desoxyribonucleic acid, which in minute amounts will induce the transformation of R variants of Type II to smooth, encapsulated, virulent Type III cells.

A similar example of the acquiring of a new immunologic character by the typhoid and paratyphoid A bacilli has been reported by Holtman.[44] These organisms could be made to acquire the ability to form heterophile antigen (p. 246) by growing them in the presence of this substance in laboratory media or by growth in a collodion sac in the peritoneal cavity of the guinea pig. In contrast to the artificial pneumococcus types, the acquired ability was not permanent but was lost after twenty to fifty transfers on ordinary laboratory media. In this connection it is of interest to recall that many years ago Smith and Reagh[45] suggested that bacteria might be immunologically altered by residence in different hosts. They were unable to demonstrate such differences, however, and accounted for the failure by the high degree of adaptation to the host of the forms studied.

The Interpretation of the Phenomena of Variation.- The significance of the phenomena of bacterial variation to biology is uncertain. Because of unique environmental factors interpretation of the morphological variation of individual cells presents unusual difficulties, and neither the mechanisms underlying this type of variability nor its relation to variation in anatomical structures of higher organisms are apparent as yet. Variation in colonial morphology, however, appears to be associated, in part, with physiological and immunological variation, and it is not unlikely that changes in colony form arise as a consequence of alterations in the nature of the cell surface.

The majority of the physiological variations are probably fluctuating variations which may be selected for, though in some instances true mutation appears as a possibility that can neither be ruled out nor unequivocally established. The significance of the apparent loss and gain of physiological characters, even if these be absolute in that a property is

[40] Griffith: Jour. Hyg., 1928, *27*:113.
[41] Dawson and Sia: Jour. Exp. Med., 1931, *54*:681.
[42] For other developments see Alloway: Jour. Exp. Med., 1932, *55*:91; *ibid.*, 1933, *57*: 265.
[43] Avery, MacLeod and McCarty: Jour. Exp. Med., 1944, *79*:137.
[44] Holtman: Jour. Immunol., 1939, *36*:405, 413.
[45] Smith and Reagh: Studies from the Rockefeller Inst., 1904, *1*:270.

completely lost or absent, is difficult to assess in general biological terms. As Needham[46] has pointed out, although a structure which makes possible a given physiological function may not be regained after being lost, the organism may re-acquire the function through the development of another and different structural mechanism. It is clearly open to question as to whether an enzyme system lost or gained by a bacterium may be regarded in the same category as an anatomical structure such as a tooth.

The immunological characters of bacteria, however, have their counterparts among the higher organisms in which immunological characters are known to be inherited, as in the case of human blood groups, or parallel phylogenetic relationships of accepted zoological classifications, as in the immunological relationship of blood proteins. In this connection it has been shown[47] that the immunological character of serum proteins of pigeons is genetically determined. If immunological characters of bacteria are to be regarded as fundamentally significant ones, it appears that on the one hand bacteria are unstable in this respect, *viz.*, the constant fluctuation of specific and non-specific flagellar antigen in the diphasic *Salmonellas*, and on the other, that such characters may be acquired through environmental influence. The fluctuating variation between specific and non-specific H antigens and the random redistribution of these antigens in the bacterial population following selection may well indicate, however, that some, at least, of these immunological characters may not be of fundamental biological importance, a suggestion that is, in part, supported by present knowledge of common antigens and partial antigens.

This apparent acquisition of new immunological characters is also of interest in a somewhat different connection, that of the apparent autocatalytic properties of some substances. Recent studies have indicated that some of the filterable viruses are proteins. Since these agents are able to increase in the body of the host, it would appear that they stimulate the host cells to form more virus protein. The analogy to the transmutation of pneumococcus types and the acquiring of the heterophile antigen by the typhoid and paratyphoid A bacilli is obvious. If the presence of capsular polysaccharide in the form of dead pneumococci or appropriate extracts in the one case, and heterophile antigen in the other, stimulates the bacterial cell to produce more of these substances, a phenomenon of profound biological significance has been demonstrated.

Bacterial Phylogeny.—From the evidence of comparative bacterial physiology (Chapter 5) it appears not unlikely that bacteria were among the first living organisms on the earth. The autotrophic bacteria may plausibly be regarded as primitive forms of life that could exist in an environment containing no organic matter, and that have persisted until the present time. Teleologically, it would seem that in the bacteria nature tried a variety of energy-yielding mechanisms ranging from the oxidation of inorganic compounds of nitrogen, carbon, sulfur, iron, manganese, etc. to the utilization of radiant energy by the photosynthetic forms. The last

[46] Needham: *Contributions of Chemical Physiology to the Problem of Reversibility in Evolution*. Biol. Rev., 1938, *13*:225.

[47] Cumley, Irwin and Cole: Proc. Nat. Acad. Sci., 1941, *27*:565.

process, however, became of quantitative importance in the green plants rather than the bacteria. With the accumulation of organic matter, transitional forms, persisting today as the facultative autotrophes, arose in which there appeared mechanisms for the oxidation of organic compounds; concurrent with this development came the ability to respire in the absence of molecular oxygen. The wide variety of bacteria which exist at the present time may well be regarded as a consequence of the physiological expansion made possible by the accumulation of a variety of organic compounds and the development of respiratory mechanisms that made possible their utilization. In this connection it is of interest that vestiges of autotrophic physiology, such as the ability to oxidize hydrogen, persist in some of the heterotrophic bacteria.

With this development of respiratory mechanisms, however, a series of degenerative changes appeared, the first being the loss of the ability to reduce carbon dioxide, followed by an increasing dependence upon preformed organic compounds as building stones for protoplasm and upon preformed components of the enzyme systems concerned in respiration, such as coenzyme, thiamin and the like. Degeneration appears most marked in the pathogenic forms (possibly reaching an ultimate in the filterable viruses if, as some think, these are microorganisms completely dependent upon the host cells for enzyme systems), and it is possible that this degeneration has been accelerated through parasitism.

Parasitism.[48]—Although the ability of some bacteria to produce disease may be a purely fortuitous coincidence, *viz.*, the tetanus bacillus, many of the pathogenic forms have, through long association, become adapted to life in the body of the host to such a degree that they are unable to survive in nature or possibly even on artificial culture media or in the bodies of animals closely related to the particular host. This seems, for example, to be the case with the leprosy bacillus, which, so far as is known, is not able to grow anywhere except in the body of man and possibly of the anthropoid ape. Theobald Smith has suggested that bacteria of great pathogenic power should be regarded as incompletely adapted parasites that have not yet succeeded in establishing an equilibrium between themselves and their host. The less complete the adaptation, the more virulent the disease produced. This concept would explain the tendency of long established diseases to decrease in severity at the same time that they are becoming more prevalent.

This adaptation to a parasitic mode of existence is indicated in a variety of ways. The nutritive requirements of some of the pathogenic bacteria, for example, such as the necessity for fresh blood, ascitic fluid and the like in laboratory media, suggest an adaptation to an environment in which these or similar substances are available—the tissues of the host. The optimum temperatures for the pathogenic bacteria are, without exception, the body temperatures of their hosts; the human type of tubercle bacillus grows best at 37° C. but the avian type is presumably adapted to the higher body temperature of the bird, 41 to 42° C.

[48] *Cf.* Smith, Theobald: *Parasitism and Disease.* Princeton University Press, Princeton. 1934.

The question of whether a host may, through long association, become adapted to a parasitic bacterium to such a degree that the presence of the microorganism is either necessary or of advantage to the continued existence of the host is an open one. In certain cases the presence of a parasitic bacterium bears a significant relation to the assimilation of food by the host; such, for example, is the case with respect to the leguminous plants and the root nodule bacteria, and the herbivorous animals and cellulose-decomposing microorganisms. Whether the abundant intestinal flora of man and other animals in general functions in a similar fashion has been the theme of considerable speculation and some investigation. Nuttall and Thierfelder[49] early showed that guinea pigs removed by Cesarean section and kept in a sterile environment survived for ten days or so, but Schottelius[50] was not successful in rearing bacteriologically sterile chicks. Nutritional inadequacies of the chick diet apparently accounted for these results, for it was later shown[51] that chicks could be raised for as long as forty days in a bacteria-free environment. The work of Reyniers[52] and his colleagues in recent years has shown that not only chickens but a variety of higher animals such as guinea pigs, rabbits, rats, etc., may develop in the complete absence of bacteria. It appears likely, therefore, that an intestinal flora is not essential to the continued existence of some of the higher animals, but whether it may be an advantage is as yet uncertain.

[49] Nuttall and Theirfelder: Ztschr. physiol. Chem., 1895–96, *21*:109.
[50] Schottelius: Arch. Hyg., 1899, *34*:210; *ibid.*, 1902, *42*:48; *ibid.*, 1908, *47*:177.
[51] Cohendy: Ann. Inst. Pasteur, 1912, *26*:106.
[52] Reyniers: *Micrurgical and Germ-Free Techniques: Their Application to Experimental Biology and Medicine.* Charles C Thomas. Springfield, Ill. 1943.

CHAPTER 7

THE CLASSIFICATION OF BACTERIA

The classification of bacteria presents peculiar difficulties that stem more or less directly from their simplicity of structure. Since taxonomy in general rests on a morphological or anatomical basis, the relationship, phylogenetic or otherwise, of bacteria to the higher forms is difficult to define, and the interrelations of the bacteria themselves present problems to which taxonomy has, to date, offered no definite or entirely satisfactory solution. A consequence of this structural simplicity is the widespread use of physiological characters in the differentiation of the bacteria into genera and species, characters whose counterparts among the higher organisms are, in general, regarded as of but minor taxonomic importance. As has been pointed out previously, differences that are quite possibly trivial biologically are often of great practical importance and hence have come to assume a taxonomic significance that may often be entirely undeserved. In other cases the biological importance of a given characteristic or group of characteristics is unknown, and the inability to assess the significance of characters proposed as differentials has led to disagreement among bacteriologists.

Such a situation clearly does not lend itself to order and system nor, and perhaps most important of all, does it allow the development of a classification which is based upon characters of fundamental significance and which shows the biological or genetic relationship of these organisms to one another. The practical consequences are two: there is no classification of bacteria which is generally accepted in more than its primary subdivisions; and, second, the bases upon which various groups of bacteria are classified differ widely, as will appear, from one group of organisms to another. It should be emphasized, therefore, that bacterial "species" are by no means analogous to the species of the zoologist or botanist and cannot be compared directly to them.

The phenomenon of bacterial variation, discussed in the previous chapter, is clearly of no small importance in the classification of these organisms. From the general biological point of view there is but little solid ground upon which a sound taxonomic structure can be built. In the practical identification of bacteria, however, such variations are not a source of great embarrassment. As has been pointed out, the majority of bacterial variations arise as a consequence of alterations in the environment and, in a working sense, may be regarded as adaptations. The observed uniformity of the morphology and biochemical properties of the bacterial culture is due to the fact that observations of these characters are always made in the same way. It is only when the procedure is varied, either in terms of the past history of the bacterial culture or in the way in which the test is conducted, that variability becomes apparent and the illusory nature of the supposed constancy of these organisms is demonstrated.

The question of whether bacteria should be regarded as members of the plant or animal kingdoms is no longer considered of great importance. Possessing characteristics of both, these organisms may be regarded as a connecting link between the plants and animals. Using the term bacteria in its broad sense, the microorganisms included under this head make up a continuous series of types which appear to connect the two kingdoms. The so-called higher bacteria, the sheathed, filamentous forms, are closely akin to the fungi; bacteria such as the tubercle bacillus and the diphtheria bacillus appear to be further removed from the plants but show some fungoid characters, such as tendencies to branching and filament formation. Among the well-known forms such as the streptococci, staphylococci, sporulating rods and gram-negative intestinal forms, the morphological relations to the fungi are less apparent; organs of locomotion appear, and with the spiral forms the bacteria merge into the protozoa. Physiological characters and the evidence of chemical composition of the cells tend to reinforce this intermediate position. The metabolism of these organisms is chemosynthetic, an animal-like character, but the ability to utilize ammonium salts as a source of nitrogen suggests a plant type of physiology; nucleic acids found only in plant cells or only in animal cells have all been found in the bacteria. It was proposed many years ago that a third kingdom be created for these organisms, the *Protista*, but since the dividing lines between such a third group and the plants on the one hand and the animals on the other are as vague as that between the plants and animals, the proposed new kingdom would solve no difficulties and has never been accepted. It is generally agreed, however, that the sum total of bacterial characters allies them more closely to the plants than to the animals, and they are classified with the plants as *Schizomycetes* or fission fungi (German *Spaltpilzen*). The relationship of the *Schizomycetes* to other plants may be indicated as follows:

Phylum I. *Thallophyta*, plants without distinction of root, stem and branch
- Subphylum 1—the algae
- Subphylum 2—the fungi—thallophytes lacking chlorophyl
  - Class I. *Schizomycetes*—the bacteria
  - Class II. *Saccharomycetes*—the yeasts
  - Class III. *Phycomycetes*—the algae-like fungi
  - Class IV. *Ascomycetes*—the fungi forming ascospores
  - Class V. *Basidiomycetes*—the fungi forming basidiospores

Phylum II. *Bryophyta*—the mosses
Phylum III. *Pteridophyta*—the ferns
Phylum IV. *Spermatophyta*—the seed-bearing plants

Although the interrelationships of the organisms included under *Schizomycetes* present problems which are, in many essentials, new to the taxonomist, certain "natural" groups are apparent. Most obvious of these are the morphological groups which were made use of by Migula[1] and by Lehmann and Neumann.[2] Migula's classification, though now unused, exerted a strong influence upon bacterial taxonomy and, together with that

[1] Migula: *System der Bacterien*. Jena, 1897.
[2] Lehmann and Neumann: *Atlas u. Grundriss der Bakt.* Munich, 1896. The current (7th) edition of this work was published in 1927 and is available in an English translation.

of Lehmann and Neumann (which is current at present in a more complex form), has been the basis upon which a great number of bacterial species have been named. The essentials of these classifications are as follows:

Migula's classification:

Order I—*Eubacteria*

- I. *Coccaceae*—spherical forms
  1. *Streptococcus*—dividing in one plane—non-motile
  2. *Micrococcus*—dividing in two planes—non-motile
  3. *Sarcina*—dividing in three planes—non-motile
  4. *Planococcus*—dividing in two planes—motile
  5. *Planosarcina*—dividing in three planes—motile
- II. *Bacteriaceae*—rod forms
  1. *Bacterium*—non-motile
  2. *Pseudomonas*—motile with monotrichous flagella
  3. *Bacillus*—motile with peritrichous flagella
- III. *Spirillaceae*—curved forms
  1. *Spirosoma*—non-motile
  2. *Microspira*—motile with one or two polar flagella
  3. *Spirillum*—tuft of polar flagella
  4. *Spirochaeta*—flexible spiral form
- IV. *Chlamydobacteriaceae*—sheathed filamentous forms
  1. *Chlamydothrix*—unbranched, uniform filaments
  2. *Crenothrix*—unbranched filaments swollen at one end, thick sheath
  3. *Phragmidiothrix*—as *Crenothrix* but thin sheath
  4. *Sphaerotilus*—branched filaments

Order II—*Thiobacteriales* (including the colorless sulfur bacteria which are divided into a number of species).

Lehmann and Neumann's classification:

- I. *Coccaceae*—spherical forms
  1. *Streptococcus*—in chains
  2. *Sarcina*—in packets
  3. *Micrococcus*—scattered or irregularly clustered
- II. *Bacteriaceae*—rod forms
  1. *Bacterium*—without spores
  2. *Bacillus*—with spores
- III. *Spirillaceae*—curved forms
  1. *Vibrio*—slightly curved, one or two polar flagella
  2. *Spirillum*—rigid spirals with lophotrichous flagella
  3. *Spirochaeta*—flexible spirals, no flagella

Appendix of "mold-like" bacteria:

1. *Corynebacterium*—rods, club-shaped, non-motile, non-spore-forming, not acid-fast (diphtheria bacillus, etc.)
2. *Mycobacterium*—acid-fast rods (as tubercle bacillus)
3. *Actinomyces*—long threads showing branching.

Morphology is, however, not a sufficient basis for the separation of bacterial species, for many morphologically similar organisms may be quite different in other respects. Physiological differences, generally readily determinable in the laboratory, have been widely used. In fact, one of the early

classifications, that of Orla-Jensen,[3] depended upon nutritional differences for the primary subdivision into three main groups:

Orla-Jensen's classification:

1. The autotrophic bacteria which, like the green plants, require neither organic carbon nor organic nitrogen compounds
2. Bacteria which require organic carbon compounds but can dispense with organic nitrogen, using ammonium salts or other inorganic compounds of nitrogen
3. Bacteria which, like the higher animals, require both organic carbon and organic nitrogen compounds

From these early beginnings bacterial classification has evolved through some sixty odd schemes to perhaps half a dozen current today.[4] The criteria made use of are, in the order of approximate fineness of distinction made:
(1) morphology—both gross and microscopic,
(2) physiology—biochemical properties,
(3) pathogenicity—of the disease-producing bacteria,
(4) immunology—the antigenic structure of the bacterial cell.
Of these current classifications those of Lehmann and Neumann,[2] Janke,[5] Pribram,[6] Kluyver and van Niel,[7] and Bergey,[8] should be mentioned. Bergey's classification, which is the most ambitious and detailed scheme yet attempted, developed from the earlier classification of Chester[9] and the work of a committee of the Society of American Bacteriologists, but is not, as is sometimes supposed, the official expression of the views of the Society as a whole. The Bergey classification has gained ascendancy in this country in recent years, though it is seldom used outside the United States. This is regarded by many as an unfortunate circumstance, and the classification has been severely criticized[10] because it is based upon arbitrarily chosen differential characters and does not rest upon a sound phylogenetic basis.

According to the most recent classification in Bergey's Manual (1939) seven orders of Schizomycetes are recognized:

1. Eubacteriales: Comprises the majority of the common and most familiar bacteria: The staphylococci, streptococci and sarcinae; the aerobic (*e.g.*, anthrax bacillus, hay bacillus) and anaerobic (*e.g.*, tetanus bacillus, gas gangrene bacillus) spore-forming bacilli; the large group of colon-typhoid-paratyphoid-dysentery bacilli; the plague bacillus, many plant pathogens, the nitrogen-fixing bacteria, the autotrophic nitrifying bacteria, a wide variety of chromogenic bacilli, the cholera vibrio and many others. *The members of this order do not show true branching.* Some are non-motile, some *motile through the agency of flagella.*
2. Actinomycetales: *These are mold-like organisms with a decided tendency to true branching. They are non-motile.* The familiar bacteria of this order are the tubercle

[3] Orla-Jensen: Centralbl. f. Bakt., Abt. II, 1909, *22*:305.
[4] For a discussion of bacterial classification and its development see Buchanan: *General Systematic Bacteriology*. Williams & Wilkins Company. Baltimore. 1925.
[5] Janke: Oesterr. Bott. Ztschr., 1929, *78*:108.
[6] Pribram: *Klassification der Schizomyceten* (*Bakterien*). Leipzig and Wien. 1933.
[7] Kluyver and van Niel: Centralbl. f. Bakt., Abt. II, 1936, *94*:369.
[8] Bergey: *Manual of Determinative Bacteriology*. 5th ed. Williams & Wilkins Company, Baltimore. 1939.
[9] Chester: *Manual of Determinative Bacteriology*. Macmillan. New York. 1901.
[10] *Cf.* Stanier and van Niel: Jour. Bact., 1941, *42*:437.

bacillus, the diphtheria bacillus, certain fungus-like organisms causing lumpy jaw in cattle (occasionally transmitted to man) and the tropical affection called "Madura foot" in man.

3. Chlamydobacteriales: Mold-like organisms with *sheathed filaments. No true branching,* but false branching in many species. *Sheath frequently impregnated with iron but no sulfur granules in the cells.* These are the "iron bacteria." None of this order is known to be pathogenic.

4. Caulobacteriales: *Non-filamentous organisms growing characteristically on stalks of gum, ferric hydroxide or other material secreted from one side or one end of the cell.* In some species the stalks may be very short or absent. *No sheaths. Typically water forms,* none is known to be pathogenic.

5. Thiobacteriales: Filamentous organisms, but *no sheaths.* These are the "sulfur bacteria." *They contain sulfur granules and certain pigments (bacteriopurpurin) in the cell.* No pathogens known.

6. Myxobacteriales: *Mold-like slimy organisms with an ameboid stage (pseudoplasmodia) which passes over into a resting stage.* No pathogens known.

7. Spirochaetales: Protozoan-like organisms. Slender flexible spirals, *motile by means of undulating cell movements and not by flagella* (the spirally-shaped cholera vibrio and related organisms form flagella). *Multiplication by transverse fission.* The organisms causing syphilis, the relapsing fevers and infectious jaundice are spirochetes.

These orders of *Schizomycetes* are fairly well defined and are recognizably useful divisions. Not so much can be said, however, of the attempted subdivisions within the order of *Eubacteriales,* the largest and most heterogeneous of the main groups. The recent creation of new subdivisions establishes twelve "families," fifteen "tribes" and fifty-eight "genera." Some of the criteria for genera are not generally accepted, and the number of "species," "varieties," "races," and "types" is legion. It is here that the greatest difficulties in the classification of bacteria are encountered.

Nomenclature.—Throughout the development of bacteriology names have been given these organisms in a haphazard fashion and often with singular disregard for the conventions of botanical nomenclature. In general, however, a bacterium has a generic name, always written with a capital letter, which may or may not be descriptive (for example, *Bacillus*—a small rod; or *Pasteurella*—in honor of Pasteur) and a specific name which may be an adjective (*albus*—white) or a noun indicating possession (*Clostridium welchii*—Welch's clostridium) or a noun in apposition (*Bacillus radicicola*—the root-dweller bacillus). The practice of indicating the author of the name, as in *Bacillus subtilis* Cohn, is not as common in bacteriology as in zoology or botany. The use of trinomial and quadrinomial names such as *Granulobacillus saccharobutyricus mobilis nonliquefaciens* is obviously highly undesirable. Trinomials are occasionally useful in the designation of varieties of subspecies. Common names are, of course, used; terms such as Friedländer's bacillus, the typhoid bacillus, etc., are frequently encountered.

Genera.—Perhaps one of the greatest difficulties the bacterial taxonomist labors under is the paucity of genera. The question of what degree of difference shall be judged sufficient to establish new genera is one to which there is as yet no satisfactory answer. At the present time considerable confusion and lack of uniformity still exist. In a number of instances new generic names have obtained wide currency among bacteriologists in many parts of the world, partly, doubtless, because they are applied to fairly distinct groups of microorganisms and have genuine classificatory value.

Such are *Brucella* (for the bacilli of undulant fever of man and contagious abortion of cattle), *Salmonella* (for the paratyphoid bacilli) and *Pasteurella* (the bacilli of hemorrhagic septicemia in domestic animals and the bacilli of plague and tularemia). Without any formal international agreement, these names can be said to have won international standing. Other generic names proposed from time to time by individuals and small groups have not been so fortunate. A number of the genera recognized in Bergey's Manual have never come into general use outside the United States and even here are not employed universally. This is partly because of the doubt some bacteriologists have felt as to the generic validity of the new grouping, as *Aerobacter* or *Alcaligenes;* partly because the names proposed are cacophonous, as *Escherichia;* partly because the old names that they are intended to displace are firmly imbedded in bacteriological literature; but also in large part because some of the new names have made their début before the world of bacteriologists too suddenly, without sufficient familiarizing discussion.

In a number of instances it seems necessary to mention both old and new names whenever a microorganism is referred to. Few bacteriologists venture to use the name *Serratia marcescens* without explaining that they mean *Bacterium prodigiosum* or *Bacillus prodigiosus*, or *Gaffkya tetragenus* without the equivalent *Micrococcus tetragenus*. Similarly the adherence of bacteriologists to various taxonomic schemes gives rise to a series of names for a microorganism; the familiar typhoid bacillus may be *Bacterium typhosum, Bacillus typhosus, Salmonella typhi* or *Eberthella typhosa*. The genus *Pfeifferella* suddenly appears and in a short time as suddenly vanishes and the new genus *Malleomyces* takes its place; few know whether the sole organism in the new genus *Listerella* deserves generic rating, or how to classify the "fusiform bacteria." On the whole it would seem reasonable to move slowly in the creation of new genera and to retain the older names until a fair degree of agreement can be reached for an acceptable international usage.

For the present the bacteriologist seems to have no escape from using a double set of generic names for certain organisms, or from using the older, albeit nomenclatorially unjustifiable, names for others, while welcoming certain new and useful generic designations such as *Brucella* and *Salmonella*. Uniformity, consistency and strict adherence to the rules of biological nomenclatorial practice are perhaps far in the future.

Species.—Classification within the genus is likewise difficult, again because there seems to be no sort of unanimity of opinion among bacteriologists as to what constitute proper criteria for establishing a species. New species have been frequently set upon a single character, such as the possession of a particular antigen. Among the pneumococci antigenic differences have been made the basis of "types;" in the genus *Salmonella* they are used for "species" characters. Among the dysentery bacilli investigators have used both serological methods and biochemical reactions for establishing "species" or "groups;" neither method has proved entirely satisfactory. The alteration of specific names of some well-established species adds, in a minor degree, to the confusion. With the creation of the genus

ARTIFICIAL KEY

A rough differential outline for the tentative identification of the kinds of bacteria of special interest to the student of medicine or public health may be given here. In many instances animal inoculations and intensive serological tests are necessary for full identification.

Spherical forms

- **Gram-negative**

	Acid produced in medium containing Dextrose.	*Maltose.*
Gonococcus	+	−
Meningococcus	+	+
Micrococcus catarrhalis	−	−

- **Gram-positive**
  - **Staphylococci (in clusters)** — Many varieties with respect to pigment production, serological behavior, etc. Differentiation of varieties of little practical value.
  - **Streptococci (in chains)**
    - Hemolytic (beta) ("Str. hemolyticus").
    - Green-producing (alpha) ("Str. viridans").
    - Indifferent (gamma) ("Str. anhemolyticus").
    - No unanimity of opinion on further classification. Some bacteriologists recognize "species" based on fermentation or serological reactions or on specific toxin production.
  - **Pneumococcus** — Usually distinguished from alpha streptococci by solubility in bile.

Rod forms

- **No true branching**
  - **Non-spore-forming Gram negative**
    - Producing conspicuous pigments: A, B, and C are mostly saprophytes. B. pyocyaneus (Pseudomonas cyaneus) has some pathogenic power (p. 475). Many plant pathogens produce abundant pigment: few if any of these are known to produce disease in animals.
      - (A) Red or pink, *e. g.*, B. prodigiosus (Serratia marcescens).
      - (B) Yellow, *e. g.*, B. aurescens (Flavobacterium aurescens).
      - (C) Violet, *e. g.*, B. violaceus.
      - (D) Green or blue-green, *e. g.*, B. pyocyaneus (found in "blue pus").
    - Intestinal bacteria: Mostly found in the intestinal tract of man and the higher animals. All, with rare exceptions, ferment dextrose and other carbohydrates, mostly with gas production; the nature of the carbohydrates attacked furnishes a valuable means of differentiation. Gelatin is not liquefied by most forms.

	Dextrose.	Lactose.	Saccharose.	Indol Production.	Gelatin Liquefied.
Typhoid bacilli	*a*	−	−	−	−
Bact. coli	+	+	±	+	−
Bact. aerogenes	+	+	+	+	−
Paratyphoid bacilli (Salmonella)	+	−	−	−	−
Proteus	+	−	±	±	+
Bact. cloacae	+	−	+	±	+
Dysentery bacilli:			*Mannite*		
Shiga type	*a*	−	−	−	−
Flexner type	*a*	−	+	±	−
Sonne type	*a*	*a*	+	−	−
Schmitz type	*a*	−	−	+	−

- Brucella (Undulant fever; contagious abortion of cattle). Grows on ordinary media, but less readily than the "intestinal bacteria"; rod forms short. No appreciable acid produced in ordinary dextrose broth, alkali produced in milk.
- Friedländer's bacillus. Short, plump, gram-negative, heavily encapsulated bacilli, growing readily and luxuriantly with a mucoid growth on most culture media. Fermenting a large variety of carbohydrates with gas production.

			Pasteurella (plague bacillus; bacillus of tularemia). Grows actively and profusely on ordinary culture media. Cocco-bacillus forms. Polar staining, numerous involution forms and great abundance in buboes facilitate identification. Hemophilus (Pfeiffer's bacillus). Distinguished by its peculiar growth requirements (p. 460). B. pertussis (whooping cough) resembles Pfeiffer's bacillus, but after preliminary cultivation in blood media, it is able to grow on plain media, which is not the case with the Pfeiffer's bacillus. Unlike the latter also, it does not ferment carbohydrates, does not produce indol and does not reduce nitrates to nitrites.
	Spore-forming Gram-positive	Aerobic	B. anthracis; B. subtilis (hay bacillus).
		Anaerobic	Clostridium tetani. Clostridium welchii. Clostridium botulinum.
True branching Gram-positive	Acid-fast	Tubercle bacillus Smegma bacillus Some actinomycetes	Often appear as slender rods; real branching seldom seen except in the actinomycetes.
	Non-acid-fast	Actinomycetes Diphtheria bacillus	
ral forms	Flagellated; small rigid spirals, and more or less curved rods. Cholera vibrio and many saprophytic forms.		
	Spirochete; long flexible spirals, motile but without flagella. Some similarities to protozoa. Treponema of syphilis; spirochetes of relapsing fever; leptospira of infectious jaundice; many large saprophytic forms.		

Eberthella, for example, *typhosum* or *typhosus* became *typhi*, but the current Bergey classification returns to *typhosa* on the ground that *Eberthella typhi* suggests typhus fever rather than typhoid fever—a justification inconsistent with the continued presence of *Salmonella choleraesuis*.

The designations of the generic subdivisions are in a particularly chaotic state. The immunological types or varieties of the pneumococci are commonly characterized by Roman numerals; similar immunological types in the genus *Salmonella* are given the dignity of species and have been assigned specific names, usually place names—"London," "Berlin," "Newport." Streptococci have been distinguished on the basis of hemolytic power by letters of the Greek alphabet, while types of *Clostridium botulinum*, differentiated by toxin production, are characterized by the letters A, B and C. In point of fact, as indicated in the preceding chapter, not enough is yet known about bacteria and their life histories to establish any classification and nomenclature based on "genetic relationships" and, for some time to come, names, "types" and subdivisions will continue to conform to convenience and to current and local usage much more closely than to a consistent and uniform taxonomic system.

CHAPTER 8

THE RELATION OF BACTERIA TO DISEASE

Prior to the discovery of the causal relation of bacteria to the infectious diseases, these afflictions were regarded as an outward manifestation of the activity of some metaphysical or supernatural agency. A common belief, and one that still persists among some primitive peoples, was that of demoniacal possession, the invasion of the human body by the demon, taking the form of punishment for misdeeds or a consequence of individual failure to take adequate precautions against malignant spirits. When the advance of knowledge brought a larger measure of understanding of the structure and functions of the human body, a new and semiscientific theory of disease sprang into being and, although not entirely displacing the concept of demoniacal invasion, attained world-wide influence. The Hippocratic theory of disease, as it was called after its founder, the Greek physician Hippocrates, postulated four bodily humors, blood, phlegm, yellow bile and black bile. Health consisted of a proper mixture of these humors, disease an improper mixture. Although supplanted to a mild degree in the seventeenth century by more complex and mystical theories such as the homeopathy of Hahnemann, the Hippocratic doctrine of humors was dominant throughout the middle ages and even yet colors much medical thought and practice.

Amid the vagueness and confusion of these half mystical hypotheses emerged the tangible and definite germ theory of disease. As already pointed out, the germ theory of disease is the legitimate offspring of the germ theory of fermentation, and owes its origin to the memorable investigations of Louis Pasteur. The belief that the infectious diseases are caused not by demons or improper mixtures of humors or by any spiritual dynamic derangement but by microscopic plants and animals is now securely established on a firm experimental foundation.

Koch's Postulates.—The unequivocal proof of a suspected causal relation between a given bacterium and a particular disease is dependent upon the development of a logical chain of experimental evidence which is often formalized as a series of postulates. These are commonly known as Koch's postulates although there is no evidence that Koch himself expressed these experimental steps in terms of formal "postulates." There are four of these:

(1) The bacterium must be observed in every case of the disease.
(2) The bacterium must be isolated and grown in pure culture.
(3) The bacterium, in pure culture, must, when inoculated into a susceptible animal, give rise to the disease.
(4) The bacterium must be observed in and recovered from the experimentally diseased animal.

Clearly, if each of these steps can be carried out, the evidence implicating the bacterium as the causative agent of the disease is very strong indeed.

Although in a great many cases this chain of experimental evidence can be developed, or, as sometimes said, Koch's postulates can be fulfilled, and the story is in its essentials complete, in a considerable number of diseases one or more of these steps cannot be carried out. In this connection, these experimental steps are best considered one by one.

The *first postulate* may be fulfilled in practically all cases if the term "observe" may be taken in its broad sense. Bacteria, of course, can be observed in the literal sense, but the submicroscopic filterable viruses cannot be seen with the aid of ordinary equipment. Their presence may be demonstrated, however, by animal inoculation, and it is not unreasonable to regard such a procedure as observation in a very real sense. It should be pointed out here that in the early days of bacteriology it was assumed that the bacterium under suspicion should be present only in cases of disease and not in healthy individuals. It is now well known that the corollary does not necessarily follow. A number of pathogenic bacteria such as those causing diphtheria, typhoid fever and the like, may be present in a virulent form in healthy persons who show no clinical symptoms.

The *second postulate* is, in general, somewhat more difficult to satisfy, although in most cases a causative bacterium may be isolated and grown in pure culture. Perhaps the best known example of an organism that has not been cultivated outside the body of the host is the leprosy bacillus (see, however, p. 591), and as a consequence it cannot be said with certainty that this bacterium is responsible for the disease. The ultramicroscopic viruses have not been cultivated on lifeless media and, although these agents may be shown to proliferate in tissue culture or in the developing chick embryo (p. 795), there is no positive assurance that such cultures are "pure," *i. e.*, that a virus is but a single entity and not a mixture of two or more viruses.

The *third postulate* is, in practice, probably the most difficult one to fulfill. The disease must be reproduced in a clinically recognizable form; a localized infection or a general septicemia is not sufficient although often suggestive. Since the most important diseases, from the anthropocentric point of view, are the diseases of human beings, it is necessary to find an experimental animal that will respond to the infection in the same or nearly the same manner as the human subject. A satisfactory experimental animal which complies with this requirement may be difficult to find, for phylogenetic relations are not necessarily correlated with susceptibility; monkeys, chimpanzees and the like are not, in general, better experimental animals than rabbits, guinea pigs, etc. The importance of the experimental animals to the study of an infectious disease is obvious; epidemic influenza, for example, in spite of the tremendous amount of concerted study which followed the 1918 epidemic, was but poorly understood until it was discovered (1933) that the disease could be reproduced in the ferret. The difficulties sometimes encountered in the experimental reproduction of a disease very likely stem in part from the adaptation of the bacterium to a parasitic mode of existence in conjunction with a particular host species.

The *fourth postulate* generally offers no difficulties if the preceding three can be satisfied. Its importance should, however, not be minimized, for the

presence of the microorganism in the experimentally infected animal is indicative of its proliferation and invasion of the host tissues.

A fifth postulate is sometimes added to the original four; namely, the injection of the products of a bacterium should give rise to the clinical symptoms of the disease. This postulate is applicable to those organisms which form soluble or exotoxins, such as the diphtheria and tetanus bacilli, but it is not of general significance in the incrimination of a bacterium as a cause of disease.

As indicated above, not infrequently one or more of these postulates cannot, or has not, been satisfied with respect to a given disease. The question then arises as to whether the suspected microorganism may be held responsible for the disease on the basis of only partial compliance with Koch's postulates. There is, unfortunately, no general answer to this; each case must be considered on its own merits. In some diseases, such as leprosy, the actual evidence consists in the observation of an association of a particular bacterium with a certain clinical syndrome and pathology. Whether the association may be interpreted in terms of causality is, of course, quite uncertain and consequently the etiology of leprosy is, as noted above, not definitely known. The absence of other possible etiologic agents may be regarded as favorable, though weak, ancillary evidence, and *Mycobacterium leprae* is generally, though tentatively, considered to be the cause of this disease.

In other cases indirect evidence assumes a more important position; in that of the virus diseases, for example, while there is no absolute assurance that a tissue culture represents a pure culture of virus, the homogeneity of the infective agent is rendered highly probable by facts such as the immunological relation existing among strains of a virus (*e. g.*, influenza or poliomyelitis) and the relative constancy of the epidemiology and pathology of the disease. The inability to isolate a filterable virus in "pure culture," then, is not a serious deterrent to the implication of these agents in the causation of disease.

The case of typhoid fever is somewhat different, for here, although the organism may be grown in pure culture, the clinical picture of the disease cannot be reproduced in the laboratory animal. Indirect evidence such as the epidemiology of the disease, the undoubted efficacy of immunization with killed suspensions of the typhoid bacillus in pure culture, etc., is so strong that the disease may be regarded as almost certainly caused by this microorganism, even in the absence of the inadvertent laboratory infections of human beings which have supplied the missing link in the chain of evidence.

It is clear, then, that while the fulfillment of Koch's postulates is, in general, essential to the proof of the bacterial etiology of a disease, in some instances in which a part of the necessary direct evidence is lacking the weight of ancillary evidence may be sufficient to make the postulated causal relation highly probable or, occasionally, practically certain. It may be noted at this point that the logical processes contained in Koch's postulates are by no means confined to the study of infectious disease; the responsibility for a given fermentation, for nitrogen fixation, etc., is fixed upon a particular microorganism by essentially the same logical development of experimental evidence.

Even though a bacterium has been shown to be pathogenic, *i. e.*, capable of producing disease, the outcome of a chance contact between such a microorganism and a prospective host is variable. The result of this contact may be negative with no clinically apparent departure from the normal physiological state regarded as health, or obvious disease of varying degree of severity may ensue. The nature of the outcome is dependent upon the interaction of two factors, the virulence of the organism and the resistance of the host. Both virulence and resistance are vague terms which express a combined effect of a variety of component factors. Some of these are known in each case, although the manner in which they function is generally not well understood, and there are undoubtedly many more which are unknown and possibly unsuspected at the present time.

VIRULENCE

The precise meaning of the term virulence is difficult to define in an entirely satisfactory fashion. Some workers include the ability to form soluble toxins as a part of the virulence of a bacterium; toxigenic diphtheria bacilli, for example, are said to be "virulent" while the atoxigenic diphtheroids are regarded as "avirulent." Others assume virulence to be synonymous with invasiveness, the ability of a microorganism to invade the tissues of the host. Under the circumstances it is perhaps best to disregard these more subtle distinctions and to define virulence as the ability of a bacterium to produce disease, thereby making virulence and pathogenicity synonymous.

The Measurement of Virulence.—By definition virulence can be measured only in terms of the production of disease. In man it is usually judged on the basis of case fatality rates, a highly virulent microorganism producing death in a large proportion of the infected persons, and one of low virulence only a small number of deaths. Similarly, the titration of virulence (or any lethal agent such as a toxin) in the experimental animal is based on deaths, but dosage, which is inversely related to virulence, can be varied. Thus virulence (or toxicity) may be defined as the dose which will produce the specified deaths in a specified animal.

In the earlier studies virulence was measured as the minimum lethal dose, defined as that amount of the substance to be tested which would just kill the experimental animal, and definitions of toxin units, etc. are given in terms of these limits (p. 256). In recent years it has become generally apparent, however, that an accurate measure is essentially statistical in nature. It is assumed, and not unreasonably, that the resistance of the normal animal to the lethal agent rests on a probability basis and is normally distributed, *i. e.*, is described by the Gaussian frequency distribution. If this function is integrated, an S-shaped curve is given by the plot of the integral which describes accumulated deaths plotted against dosage. The point of inflection is, of course, that dose with which 50 per cent of the animals die. This is called the *median lethal dose* or LD_{50} dose. This dose can be calculated by a relatively simple procedure[1] from the accumulated deaths from graded doses extending through the 50 per cent endpoint.

[1] Reed and Muench: Amer. Jour. Hyg., 1938, *27*:493.

For purposes of comparison between, for example, normal and treated animals, determination of the LD_{50} doses allows a relatively precise measure having a standard basis. A somewhat less satisfactory but comparable procedure is that of relative survival times. A variety of other methods of analysis may be used for purely comparative purposes. For example, control and experimental death rates may be tested for statistical significance by the point binomial or fourfold table. Comparative death ratios, such as 8/10 to indicate that 8 animals died of 10 inoculated, are almost useless from the quantitative point of view.

Toxins.—It is difficult if not impossible to account for the derangement of the normal physiological processes and attendant clinical symptoms of an animal suffering from an infectious disease on the basis of a purely mechanical effect of the presence of the invading bacteria in the blood or other tissues. It was early discovered that a number of the pathogenic bacteria produce poisonous substances which, when absorbed by the host, give rise to the symptoms and pathology characteristic of the particular disease. It has been pointed out previously (p. 129) that these poisonous substances or toxins fall into two categories: (a) the *soluble toxins*, *exotoxins* or *true toxins* which apparently diffuse out of the intact bacterial cell into the surrounding culture medium or tissue; and (b) the *endotoxins* which do not diffuse out of the intact bacterial cell but may be separated from it *in vitro* by mechanical disruption of the cell structure. These substances are liberated *in vivo* through destruction of the bacterial cells by the various defensive mechanisms of the host.

The soluble toxins, although not a part of the cell substance, cannot be regarded as secretions in the usual sense of the word. The rate of appearance of toxin does not, except in the case of *Colstridium welchii*, parallel the rate of growth of the culture; toxin is liberated in the greatest amounts after the phase of active growth is over and many of the cells are dead or dying. The endotoxins appear to be a part of the cell substance of bacteria containing them; organisms such as the cholera vibrio, certain of the dysentery bacilli and the like may be regarded as microorganisms whose protoplasm is toxic to the higher animals which they infect. There are a number of differences between these two types of toxins which may be summarized as follows:

Exotoxins	Endotoxins
(1) Occur outside the bacterial cell.	(1) Intracellular in the intact cell.
(2) Poisons of extremely high potency.	(2) Low potency.
(3) Excellent antigens which give antisera of high titer.	(3) Poor antigens, antisera of low potency.
(4) Combine with antibody according to the law of multiple proportions.	(4) Do not combine in multiple proportions.
(5) Protein in nature.	(5) Some are proteins but some are glucolipoids.
(6) Thermolabile.	(6) Thermostable.
(7) Destroyed by proteolytic enzymes.	(7) Resistant to the action of proteolytic enzymes.
(8) Detoxified by formaldehyde.	(8) Toxicity not affected by formaldehyde.

These differences are by no means absolute, and the listing of them represents a generalization to which there are a number of exceptions, as will appear. Consequently, the identification of a toxin as an exo- or endotoxin can be made, not on the basis of a single property, but only on the aggregate of the properties it exhibits. Even when all its properties are taken into consideration, a given toxin, such as the Shiga dysentery bacillus toxin, for example, may occupy an anomalous position.

The soluble bacterial toxins are, by a comfortable margin, the most potent poisons known; only the vegetable poisons, ricin and abrin, approach them in potency. In terms of minimum lethal dose (MLD) for an adult man, tetanus toxin, in an impure form, compares with other poisons as follows:

atropine	130 mg.
strychnine	30–40 mg.
cobra venom	4.4 "
tetanus toxin	<0.2 "

The tremendous potency of these soluble toxins is further indicated by the extremely small quantities of purified material necessary to kill experimental animals. Eaton's[2] and Pappenheimer's[3] purest preparations of diphtheria toxin killed guinea pigs in amounts of 0.0004 mg. per kilogram body weight. The most potent of the soluble toxins are those formed by *Clostridium tetani*, *Clostridium botulinum* and the diphtheria bacillus; others, such as that of *Clostridium welchii*, are much less potent, and it may require as much as 0.1 cc. of culture filtrate to kill an experimental animal. Still less potent are the endotoxins whose minimum lethal dose may be as much as one million times greater than that of a potent exotoxin. The MLD of a killed broth culture of the cholera vibrio, for example, is about 0.5 cc. for the guinea pig.

The potency of these toxins is, in general, paralleled by their efficiency as antigens. Antitoxic sera of high titer, 1 cc. of which will neutralize thousands of guinea pig MLD's, may be obtained against diphtheria toxin, but antitoxic sera prepared against endotoxins are generally of very low titer—several cubic centimeters may be required to neutralize 1 to 5 MLD's of endotoxin. The neutralization of exotoxin by antitoxin proceeds according to the law of multiple proportions, *i. e.*, if x units of antitoxin neutralize y units of toxin, nx units of antitoxin will neutralize ny units of toxin (see p. 257). The neutralization of endotoxin does not proceed in this orderly manner; it may be possible to protect an animal against 4 to 5 MLD's by the injection of antiserum, but if much more endotoxin is given, greater amounts of antiserum will not provide protection.

The nature of toxins, the soluble toxins in particular, has been a subject of long-continued interest.[4] The soluble toxins appear to be proteins; they are denatured by heat, may be salted out of solution, etc. None of them has been crystallized, but it is not unlikely that the preparations of Eaton[2] and Pappenheimer[3] represent pure diphtheria toxin. Both preparations

[2] Eaton: Jour. Bact., 1936, *31*:347.
[3] Pappenheimer: Jour. Biol. Chem., 1937, *120*:543.
[4] See the review by Eaton: Bact. Rev., 1938, *2*:3.

were found to contain 0.00046 to 0.00055 mg. of nitrogen per Lf unit (roughly 100 guinea pig MLD's; see p. 256). With the exception of botulinum toxin, the soluble toxins are destroyed by proteolytic enzymes; botulinum toxin is, therefore, the only toxin which is effective when given by mouth. There is no chemical evidence which explains, even in part, the toxicity of these substances; these proteins do not seem to differ in any essential respect from bland proteins, such as egg albumin. It has been suspected that toxicity might be a property of a prosthetic group attached to the protein molecule, but this appears not to be true; the balance of evidence indicates that the toxicity is a property of the structure of the toxin molecule, possibly of the arrangement of the constituent amino acid molecules in the protein. It has been suggested that the toxins may act by blocking some essential metabolic process, as appears to be the case with some of the sulfa drugs (p. 72), or they may function as enzymes which destroy some essential structure in susceptible cells or distort normal cellular physiology to produce abnormal metabolic products.[5]

The discovery that the toxic qualities of the soluble toxins are destroyed by treatment with formaldehyde which, at the same time, leaves the antigenic and antitoxin-combining properties of the toxin unimpaired, has been of the greatest practical importance in immunization procedures. Toxin so treated is called *toxoid* or *anatoxin*. It might be supposed that this observation would throw some light on the nature of the toxicity of these substances since formaldehyde is known to block amino groups—a fact taken advantage of in Sørensen's formol titration of amino acids. Because of the effect of formaldehyde on toxicity and the results of detoxification with ketene and phenyl isocyanate, some workers have supposed that the free amino groups of the toxin molecule, such as the ϵ-amino group of lysine, are intimately associated with toxicity, but the evidence for this is by no means complete.

The endotoxins are, in general, much more resistant to heat and to the proteolytic enzymes than are the soluble toxins. Many require heating to 80–100° C. for one hour, and an endotoxin isolated from the meningococcus requires one hour at 120° C. for destruction; in contrast, the most resistant of the soluble toxins, botulinum toxin, is destroyed by exposure to 80° C. for ten minutes. Some of the endotoxins are destroyed by the proteolytic enzymes, but others appear to be immune to the action of these ferments. Similarly, formaldehyde has little or no effect on the majority of the endotoxins.

Some of the endotoxins are protein, and it is thought by some that these substances are not toxic *per se* but are broken down in the animal body, either in the cells or body fluids, to toxic split products.[6] Recent work[7] has indicated that some of the endotoxins are not proteins but glucolipoids which are toxic to the animal body just as are some of the glucosides.

[5] Macfarlane and Knight: Biochem. Jour., 1941, *35*:884.

[6] Vaughn, Vaughn and Vaughn: *Protein Split Products in Relation to Immunity and Disease.* Lea and Febiger. Philadelphia. 1913.

[7] Boivin and Mesrobeanu: Rev. Immunol., 1935, *1*:553; *ibid.*, 1936, *2*:113; *ibid.*, 1937, *3*:319.

Substances such as these would not, of course, be affected by formaldehyde or proteolytic enzymes. Their toxicity is destroyed by mild acid hydrolysis.

Following the injection of exo- or endotoxins, a period of incubation elapses before symptoms of intoxication appear. This incubation period may be very short or may extend to thirty-six or forty-eight hours or longer. It may not be reduced below a lower limit, however; the usual incubation period of tetanus toxin is thirty-six hours and although this may be reduced to twelve hours by the injection of large amounts of toxin, it cannot be reduced below this point no matter how much toxin is administered. It is sometimes stated that endotoxins have no incubation period, thereby differing from the exotoxins, but this is not generally true.

The pharmacological action of the soluble toxins resembles that of the vegetable alkaloids and is sometimes quite definite and characteristic for each toxin. Diphtheria toxin, for example, produces degeneration of the heart muscles, kidneys and liver and the hemorrhagic reaction in the adrenals which is a highly characteristic postmortem finding in the guinea pig. A part of tetanus toxin (tetanospasmin) has an affinity for the motor nerves. On the other hand, the symptoms and pathology resulting from administration of the endotoxins are not at all characteristic although minor distinctions have been reported. Animals inoculated with lethal doses of these substances usually show dyspnea, diarrhea and, in some cases, flaccid paralysis of the posterior extremities, become progressively weaker and finally die without having exhibited symptoms that might be regarded as characteristic of a particular endotoxin. The histopathology often indicates that the toxin exerts its effects on the blood vessels, damage to which results in degenerative changes in the tissue supplied.

It is probable that there are certain "nutritive requirements" for the formation of toxins. It is, for example, quite possible to grow a toxigenic bacterium on a medium of simplified composition, good growth being accompanied by the concurrent formation of either no toxin or a very weak toxin.[8] Complex organic compounds are probably not essential in all cases, but other substances may be required; toxin production by the diphtheria bacillus, for example, occurs in simple synthetic media in the presence of both copper and iron in optimal amounts but not in the absence of those elements.[9]

As has already been suggested, the bacterial toxins are not unique. Substances closely resembling the soluble toxins occur in the seeds of some of the higher plants and in the secretions of certain animals. Among the better known toxins of plant origin (phytotoxins) are ricin (from the castor oil bean, *Ricinus communis*), abrin (from the jequirity bean, *Abrus precatorius*), and the similar substances, crotin and robin. The more familiar examples of similar poisons of animal origin (zootoxins) include snake

[8] *Cf.* Burrows: Jour. Inf. Dis., 1933, *52:*126.

[9] Locke and Main: Jour. Inf. Dis., 1931, *48:*419; Pappenheimer and Johnson: Brit. Jour. Exp. Path., 1936, *17:*335, 342; Pappenheimer, Mueller and Cohen; Proc. Soc. Exp. Biol. Med., 1937, *36:*795. See also the general review by Bordet: Ann. Inst. Pasteur, 1938, *61:*618.

venoms, the poisons of scorpions and spiders and an actively poisonous substance present in eel blood. The chemical behavior and physiological action of these poisons is strikingly similar to those of the bacterial exotoxins. Antitoxins have been prepared against a number of these substances and the antivenins have been used with considerable success.

The "Invasiveness" of Bacteria.—It was early suggested that, in addition to toxins, some of the highly virulent bacteria which invade the host tissues with great rapidity are enabled to do so by the secretion of soluble substances termed *aggressins* by Bail[10] and *virulins* by Rosenow.[11] Although subsequent experiment has provided evidence which strongly supports this general concept, these terms have, at present, little more than historical significance. It has been found that virulence in terms of tissue invasion and destruction of the defensive mechanisms of the host by bacteria may be subdivided into a number of component factors. The more important of these are the *hemolysins* and *leucocidins* which destroy the red and white blood cells, the *coagulases* and *fibrinolysins* which influence the formation and dissolution of blood clots and a *spreading factor* which produces a marked increase in the permeability of the host tissues. Although some of these properties are associated with virulence, others are not so correlated and their relative importance is uncertain. It is probable that the ability of a bacterium to invade the tissues is not dependent upon any single property but rather upon a combination of properties, including both those that are known and others that are as yet unknown.

Hemolysins.—A variety of bacteria produces hemolysins, substances which bring about the dissolution of the red blood cells of higher animals. The bacterial hemolysins, which are to be distinguished from the immune hemolysins formed by an animal in response to the injection of red cells of another species (p. 259), are of two types, the so-called filterable hemolysins which are extra-cellular and may be separated from the bacterial cells by filtration, and the hemolysins which are demonstrated by the cultivation of bacteria on semisolid media containing whole blood.

The *filterable hemolysins* are sometimes named after the bacteria which form them; a *streptolysin*, for example, is a hemolysin produced by streptococci, and a *staphylolysin* a hemolysin of staphylococci. Hemolytic activity is demonstrated by the addition of filtrate or whole culture to a suspension of washed erythrocytes in physiological salt solution; after a period of incubation the red cells are laked and hemoglobin appears free in solution. The relation of these hemolysins to other naturally occurring hemolysins such as saponins, the hemolysins present in snake venoms and the like, is uncertain.[12] The bacterial hemolysins appear to be protein in nature, are inactivated by heating (55° C. for thirty minutes), and are antigenic, *i.e.*, when injected into animals they stimulate the formation of antihemolysins.

[10] Bail: Arch. Hyg., 1905, *52*:272.

[11] Rosenow: Jour. Inf. Dis., 1907, *4*:285.

[12] For a general discussion of hemolysis see the exhaustive monograph of Ponder: *The Mammalial Red Cell and the Properties of Hemolytic Systems*. Protoplasma Monograph, No. 6, 1934.

That hemolytic activity is a property of a group of substances of bacterial origin rather than of a single substance formed by a number of bacterial species is indicated not only by differences in immunological specificity but also by the varied properties of the activity. Many hemolysins, for example, are oxygen-stable, but some, produced by the pneumococcus and certain strains of streptococci, are oxygen-sensitive, *i.e.*, they are active in the reduced form but inactive in the oxidized form, the oxidation-reduction being reversible at low temperatures.[13] Hemolysins further differ from one another in their heat and acid resistance and in the incubation time which precedes visible laking of the red cells. Differences in their activity on the erythrocytes of various species of higher animals may be marked; a given hemolysin, for instance, may lyse sheep cells but not rabbit

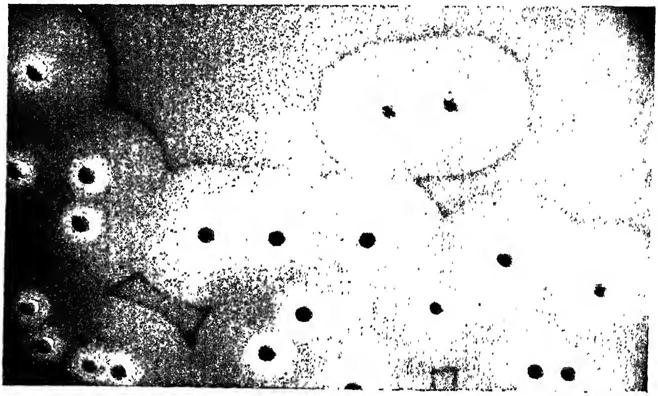


Fig. 23.—"Hot-cold" hemolysis. *Staphylococcus aureus* on sheep blood agar. Produced by holding the plate alternately at incubator and refrigerator temperature. The small clear zones are produced in the incubator by the α lysin and the large zones in the refrigerator by the β lysin.

cells. In general the cells of the animal species from which a bacterium is isolated are more sensitive to its hemolysins than are the cells of other animal species.

A single bacterial strain may form more than one hemolysin. Certain strains of staphylococci form at least two hemolysins, one acting on both rabbit and sheep cells and bringing about a rapid lysis at 37° C., and the other acting only on sheep cells which are lysed after holding at room temperature over night—a phenomenon which has been termed "hot-cold" lysis.[14] Similarly, some strains of streptococci produce two hemolysins, one of which is relatively heat-stable but oxygen-sensitive and the other oxygen-stable but heat-sensitive.[13] The former is termed α lysin and the latter β lysin. The action of both can be shown in culture on sheep blood agar, the α lysin producing a zone of complete clearing on twenty-four hours' incubation and the β lysin producing a zone of darkening which becomes

[13] *Cf.* Todd: Jour. Path. and Bact., 1934, *39*:299.
[14] Glenny and Stevens: Jour. Path. and Bact., 1935, *40*:201.

lighter and clear on subsequent refrigeration of the plate.[15] This phenomenon is illustrated in Fig. 23.

The production of hemolysins is favored by inclusion of serum in the broth in which the microorganisms are cultured, and it is of some interest that the activity of the hemolysin formed in such media is most marked on the red cells of the animal species from which the serum was derived.[16] The hemolytic activity of a culture may be transitory, probably because of inactivation of formed hemolysin. Some hemolysins lose their activity when incubated at 37° C. for two hours or more; for example, de Kruif and Ireland[16] found a maximum hemolytic titer after eight hours' incubation of streptococcus cultures which declined rapidly thereafter so that in many cases no activity could be detected in filtrates of fourteen-hour cultures.

Blood-Plate Hemolysis.—The colonies of some bacterial species on blood agar produce visible changes in the medium immediately surrounding the colony which are termed "hemolysis." Two general types of change are observed, one designated as α or green hemolysis, in which the bacterial colony is surrounded by a zone of greenish discoloration, and the other, β-hemolysis, in which the zone around the colony is clear and uncolored, in constrast with the red opacity of the medium. According to Brown[17] the zone of discoloration of α-hemolysis is surrounded by a very narrow clear zone, but this is not apparent except upon careful examination. Microscopic examination of the green zone shows the presence of many discolored corpuscles, but in β-hemolytic zones corpuscles cannot be found.

The relation between blood-plate hemolysis and the filterable hemolysins is usually regarded as uncertain, for, although microorganisms hemolytic on blood plates frequently do not appear to produce filterable hemolysin, some workers maintain that, since filterable hemolysins are difficult to demonstrate, failure to find them is not significant. It would appear that the processes involved are different; in the case of the filterable hemolysins the permeability of the red cell is altered and the hemoglobin escapes into the surrounding fluid, while in blood-plate hemolysis the pigment is broken down to green or colorless compounds. In the case of the cholera vibrio the two processes have been sharply differentiated,[18] since the identification of this organism is dependent in part on the hemolysis of goat erythrocytes in suspension (Grieg test); though the true cholera vibrio is negative to this test, some strains show hemolysis on blood agar plates. van Loghem has distinguished between the hemolysis of goat erythrocyte suspensions and what he has termed hemodigestion on blood agar. Green hemolysis was formerly thought to be due to the formation of methemoglobin and associated with the formation of hydrogen peroxide, but recent work[19] has indicated that the green substance is an iron-containing derivative of hemoglobin very possibly formed by reduction. The mechanism of β-hemolysis is not well known, but in the case of staphylococcal hemolysins,

[15] Christie and Graydon: Australian Jour. Exp. Biol. & Med. Sci., 1941, *19*:9.
[16] de Kruif and Ireland: Jour. Inf. Dis., 1920, *26*:285.
[17] Brown: Rockefeller Institute for Medical Research, Monograph No. 9, 1919.
[18] Especially by van Loghem: see Centralbl. f. Bakt., I Abt. Orig., 1926, *100*:19.
[19] *Cf.* Anderson and Hart: Jour. Path. and Bact., 1934, *39*:465.

the hemoglobin is not destroyed; according to Christie and Graydon[15] the clearing around the colony is due to migration of the liberated hemoglobin.

As in the case of the filterable hemolysins, hemolysis on blood agar is frequently species specific; a higher proportion of bacterial strains will show hemolysis on blood from one animal species and not on that of another. Brown[17] found, for example, that of

83 strains of streptococci isolated from tuberculous sputum
  5 were hemolytic on rabbit blood agar and
  60 were hemolytic on human blood agar, and of these
    53 were hemolytic on horse blood agar,
    47 were hemolytic on sheep blood agar and
    4 were hemolytic on rabbit blood agar;
  5, the ones hemolytic on rabbit blood agar, showed filterable hemolysin.

The relation of ability to form hemolysin and virulence is obscure. Although among some of the parasitic bacteria such as streptococci and staphylococci, virulence is associated with hemolytic activity, a number of saprophytic bacteria also produce hemolysins. On the other hand, it has been reported that in staphylococcus infections of the udder, the hemolytic strains are much more irritating than the non-hemolytic organisms.[20] It might be expected that infection with actively hemolytic bacteria would be accompanied by anemia and hemoglobinuria but this is not the case, as a rule; infections with *Clostridium welchii*, however, are frequently characterized by a gross blood destruction and consequent severe anemia and jaundice which is due to the continuous release of the hemolytic α-toxin (p. 428).

Leucocidins.—A number of bacteria, notably staphylococci, streptococci and pneumococci, form leucocidins, substances which kill and, in some cases, lyse, polymorphonuclear leucocytes. The death of the leucocytes results in the inability of these cells to reduce methylene blue, a phenomenon made use of by Neisser and Wechsberg[21] and others to demonstrate the activity of these substances. Recently a direct microscopic method has been introduced[22] which has shed some light on the effect of these substances on the white blood cell. Human and rabbit leucocytes in contact with leucocidin become spherical; the nuclei fragment and the cells may, with some leucocidins, eventually burst or, with others, remain intact.

The amount of leucocidin produced by one bacterial species may vary widely from one strain to another, and a single strain may produce more than one leucocidin. The detection of more than one leucocidin is dependent upon differences in thermostability and in the kinds of leucocytes attacked; one staphylococcal leucocidin, for example, affects both rabbit and human leucocytes, while another is active only on rabbit cells. In

[20] Plastridge, Anderson, Williams and Wierether: Storrs Exp. Sta. Bull. No. 231, 1939.
[21] Neisser and Wechsberg: Ztschr. Hyg. u. Infektionskr., 1901, *36*:299.
[22] Valentine: Lancet, 1936, *i*:526. It should be pointed out that it is claimed by some workers that the leucocidin tested by one method is not the same as that tested by the other.

general, the leucocidins closely resemble the filterable hemolysins in that they are antigenic, variable in their heat resistance, etc., and, in some cases, may be identical with hemolysins. The α-hemolysin of staphylococcus, for example, is a leucocidin.[23] They may not be strain specific; some of the staphylococcal leucocidins, for example, are immunologically similar if not identical regardless of the bacterial strain from which they are derived.[24]

The part played by the leucocidins in the virulence of a bacterium is obscure. Theoretically, it should be of some advantage to an invading microorganism to be able to destroy these phagocytic cells and thereby break down, to some degree, the defenses of the body. The polymorphonuclear leucocytes (heterophils) are not, however, the most important cells in the phagocytic destruction of bacteria (p. 291), and the effect of these substances on the other phagocytic cells, the mononuclears, the histiocytes and others is not known. As in the case of the hemolysins, the leucocidins of bacterial origin must be differentiated from the immune leucocidins, those produced by the animal body in response to the injection of leucocytes from another species.

Coagulase and Fibrinolysin.—The formation of blood clots is accelerated by a substance, coagulase, formed by some bacteria, and the clots formed, either with or without the acceleration of this substance, may be dissolved by other substances of bacterial origin, the fibrinolysins. The production of coagulase is, so far as present information is concerned, confined to the staphylococci for the most part, and may be causally related to the thrombi that are common in infections with these organisms. Occasional strains of other organisms such as *Pseudomonas pyocyaneus*, *Bacterium prodigiosum*, *Bacterium coli* and *Bacillus subtilis* have, however, been observed to accelerate the clotting of blood. Coagulase activity is demonstrated by the addition of bacteria to citrated or oxalated blood plasma (usually rabbit), which gels within three hours. The nature of the mechanism of this clotting action is uncertain; some aspects of its activity, *i.e.*, the clotting of fibrinogen solutions, suggest the action of thrombin, but coagulase is not inhibited by "antithrombin" such as heparin or hirudin. The activity is not filterable and is relatively thermostable; it is only partially destroyed by exposure to 100° C. for thirty minutes. Other properties such as precipitation with alcohol and acid and ammonium sulfate suggest a protein nature. Its antigenicity is uncertain. The relationship of coagulase to virulence is obscure, although it is often said that there is no association between the two. It would seem that the property of accelerating the clotting of blood might, on the one hand, be unfavorable to further invasion of the host tissues by a microorganism forming this substance,[25] and, on the other, serve as a temporary protection against the defenses of the host.

[23] Wright: Lancet, 1936, *i*:1002.

[24] For a general study of these substances see Todd: Brit. Jour. Exp. Path., 1942, *23*: 136.

[25] For a summary of information regarding coagulase see the review by Blair: Bact. Rev., 1939, *3*:97.

Fibrinolysin.[26]—Perhaps more important to the virulence of a bacterium is the ability to dissolve formed blood clots through the agency of fibrinolysin. It has been observed that if a small amount of bacterial culture is mixed with oxalated plasma and the latter allowed to clot by the addition of calcium chloride, the fibrin clot may be dissolved within a short time as a result of bacterial activity. At present it appears that the ability to bring about this lysis is confined to certain groups of streptococci, although other bacteria such as staphylococci, the plague bacillus and others have been described as fibrinolytic. Like the hemolysins, the fibrinolysins appear to exhibit some degree of specificity with respect to the source of the fibrin; streptococci isolated from human infections, for example, will lyse clotted human plasma more rapidly than plasma clots from other animals. Fibrinolysin appears to fall into the same general category as the toxins, hemolysins and the like in that it is found in culture fluid freed from bacterial cells, appears to be protein in nature, and is antigenic. Fibrinolysin is, however, distinct from these other substances; although a high correlation has been observed between the occurrence of filterable hemolysin and fibrinolysin, in many cases a bacterial strain is able to bring about the dissolution of erythrocytes but not solution of clots, and *vice versa*. The nature of the lytic process is not well understood, but it seems established that it is not an enzymatic hydrolysis. Fibrinolysin will dissolve plasma clots within a relatively short time, fifteen minutes to four hours, while dissolution resulting from proteolysis requires at least twenty-four hours and usually longer. Furthermore, in many cases actively proteolytic streptococci are not fibrinolytic.

It should be noted here that a number of bacteria exhibit a tendency to inhibit the clotting of plasma, a property that may be demonstrated by delaying the addition of calcium to the oxalated plasma; when calcium is added after a period of preliminary incubation, the clot fails to form. This property has been most often demonstrated with cultures in dextrose broth, and in some cases, at least, the failure of the plasma to clot is a result of the presence of lactic and other acids formed in the fermentation.

Fibrinolysin appears to be closely associated with virulence and particularly with the ability to invade the body tissues. Since one of the first reactions of the body to tissue destruction is the formation of blood clots which tend to wall off and isolate the infected region, it is not surprising that a bacterium capable of lysing these clots should show marked tendencies to extensive tissue invasion. The streptococci are among the most invasive of the pathogenic forms, and this phase of their virulence may be attributed in part to the formation of fibrinolysin.

Spreading Factor.[27]—It has been found in recent years that the permeability of the tissues is remarkedly increased by a factor, often called the *Duran-Reynals factor* after its discoverer, present in certain mammalian tissues, notably the testes. Bacteria, vaccinia virus, and substances such as toxins, india ink and the like, diffuse rapidly from the site of inoculation when injected in conjunction with extracts containing this factor. Strains

[26] See the review by Tillett: Bact. Rev., 1938, *2*:161

[27] *Cf.* the review by Duran-Reynals: Bact. Rev., 1942, *6*:197.

of staphylococci and streptococci that have no pronounced invasive powers may, in this way, be rendered highly invasive. This factor is also present in a number of bacteria notable for their invasive properties, such as certain strains of staphylococci and streptococci, pneumococci and certain of the obligate anaerobes such as the bacillus of gas gangrene. Among some of these organisms there is a marked association between content of the Duran-Reynals factor and virulence; the non-invasive strains of staphylococci noted above contain little or none of this factor but become invasive when it is supplied, while the invasive strains produce the factor themselves.

It has been found[28] that there is a close relationship between the enzyme hyaluronidase and spreading factor and it seems probable that they are identical. The substrate of hyaluronidase is hyaluronic acid, a mucopolysaccharide consisting of acetyl glucosamine and glucuronic acid,[29] which acts as a cement substance of the tissues and is found in synovial fluid and elsewhere in the body. The action of the enzyme in decreasing the viscosity of hyaluronic acid by hydrolysis is responsible for facilitating the penetration of the tissues by bacteria that produce it. It is of some interest that hyaluronic acid is also found[30] in the capsular substance of some strains of streptococci. Such strains do not produce hyaluronidase and in its presence are denuded of capsules and more readily phagocytosed (see below).[31] The ability of such strains to invade the tissues does not, of course, depend upon their elaboration of hyaluronidase. The activity appears to be antigenic, in that it is neutralized by antisera, but immunologically distinct from different sources; it has been suggested that the enzyme is combined with different proteins in different organisms.

Capsules.—The remarkable association between the presence of a capsule on a pathogenic bacterium and its virulence has been discussed elsewhere (Chapter 6). The capsular material, generally polysaccharide in nature although nitrogen and amino acids may be present, is not in itself toxic and cannot be regarded as analogous to toxins, hemolysins and similar substances. Rather the capsule appears to function as a defensive mechanism of the bacterium against the phagocytic activity of the leucocytes. Encapsulated bacteria may be ingested by a white blood cell but, instead of being killed and digested, remain within the phagocyte for a time and then may be extruded in a viable condition. The ability of an encapsulated bacterium to resist phagocytic destruction may, in fact, result in a wider distribution of the microorganism than it might otherwise attain, through transport within the phagocytic cell. It is of some interest in this connection that non-encapsulated, avirulent pneumococci are highly virulent for rabbits deprived of their leucocytes.[32] It is perhaps suggestive that the polypeptide material making up the capsule of the anthrax bacillus contains *d* (–) glutamic acid[33] (p. 502). Since proteolytic enzymes attack only

[28] Hobby, Dawson, Meyer and Chaffee: Jour. Exp. Med., 1941, *73*:109.
[29] Meyer and Palmer: Jour. Biol. Chem., 1943, *107*:629.
[30] Seastone: Jour. Exp. Med., 1943, *77*:21.
[31] *Cf.* McClean: Jour. Path. Bact., 1941, *53*:13.
[32] Rich and McKee: Bull. Johns Hopkins Hosp., 1939, *64*:434.
[33] Bruckner and Ivanovics: Ztschr. f. physiol. Chem., 1937, *247*:281; Ztschr. Immunitäts., 1937, *90*:304; *ibid.*, 1937, *91*:175; Naturwissenschaften, 1937, *25*:250.

polypeptides built up from amino acids of the *l*-series, possibly an encapsulated anthrax bacillus would be highly resistant to the digestive enzymes of a phagocytic cell.

Whether the association of virulence and capsule formation may be entirely accounted for on the basis of bacterial defense against phagocytic destruction is not entirely certain, but such defense undoubtedly plays an important part. The presence of antibodies to the capsular substance breaks down this bacterial resistance, and immunization to the capsular material of the pneumococcus, for example, produces just as high a degree of immunity to pneumococcus infection as immunization to the entire bacterium.

Miscellaneous Factors.—In addition to these more or less well defined and better known factors, a number of bacteria have been reported to produce substances which may be associated with virulence. Such, for example, are the necrotizing factor, or necrotoxin, produced by some staphylococci, which kills tissue cells; a hypothermic factor produced by Shiga dysentery bacilli, which lowers body temperature; an edema-producing substance formed by pneumococci; substances associated with the endotoxins of some of the enteric bacteria which affect the blood sugar levels of animals, and so on. Unfortunately, the discontinuous character of present information does not support any satisfactory generalization regarding bacterial virulence, but it seems clear that a pathogenic bacterium may have at its disposal a series of mechanisms, in combination peculiar to itself, which make possible a successful invasion of the tissues of the host.

In this connection it should be noted that bacterial virulence may be apparently enhanced by certain substances not of bacterial origin. The presence of silica in the lungs, for example, predisposes materially to pulmonary tuberculosis; the injection of calcium salts with tetanus bacilli aids, in some unknown manner, in the establishing of a nidus of infection. The suspension of bacteria, such as meningococci or typhoid bacilli, in solutions of gastric mucin markedly enhances their virulence, or, more precisely, interferes with the defense mechanisms of the host. It has been found that in the case of the meningococcus the mucin interferes with the bactericidal action of the body fluids and, when mixed with peritoneal fluid, provides a medium for its growth.[34]

Relative Pathogenicity of Bacteria.—From the foregoing considerations it will be clear that the virulence of a bacterium is an expression of the efficacy of the aggregate of offensive mechanisms that it may possess. It is to be expected, then, that the bacteria constitute a continuous series of types ranging from those which are unable to infect higher organisms, such as the autotrophic forms, to those which produce a fulminating, widely disseminated and often fatal infection. The harmless saprophyte, *Bacillus subtilis*, for example, has been found to cause, on occasion, an eye infection in human beings. (See also p. 510.) Somewhat more pathogenic is *Clostridium botulinum*, an organism which is also a saprophyte but which produces a highly potent soluble toxin. The bacterium is itself unable to infect the animal body; the toxin is formed outside the body and gives rise

[34] MacLeod: Amer. Jour. Hyg., 1941, Sec. B, *34*:51.

to disease only when ingested in a preformed state. *Clostridium tetani* is one step higher in the scale of pathogenicity; this microorganism resembles the botulinus bacillus in that it is essentially a saprophytic form that produces a soluble toxin. In this case, however, the bacterium is able to establish a nidus of infection in the tissues, albeit in a strictly localized area, and produce the toxin whose absorption gives rise to the symptoms of the disease. The diphtheria bacillus likewise generally produces only a local infection from which its toxin diffuses into the animal body but, unlike the tetanus bacillus, does not lead a saprophytic existence in nature but must maintain a close association with its host. The highly invasive bacteria, such as certain strains of streptococci and staphylococci, are, however, disseminated rapidly throughout the body, presumably because of their armament of fibrinolysins, spreading factor and the like.

A variety of types of infection may, therefore, be distinguished. A bacterium having but small ability to spread through the tissues produces a *localized* or *focal* infection. Such, for example, are the abscesses at the roots of the teeth, the infection of the heart valves in bacterial endocarditis, etc. A local infection is not necessarily of small consequence; not only may the local tissue destruction be highly significant to the host, as in pulmonary tuberculosis, but, when the microorganism produces a soluble toxin, a poisoning or *toxemia* results, as in diphtheria or tetanus.

Many pathogenic bacteria exhibit a tendency to localize in one tissue in preference to another, sometimes termed *elective localization*; the meningococcus, for example, most frequently localizes in the central nervous system, as do the so-called "neurotropic" viruses, and some of the streptococci show similar preferences for the joints. In some cases the tendency to localize is so marked that a pathogenic bacterium is infectious by one route but not by another; microorganisms such as streptococci, pneumococci, the tetanus bacillus and others are relatively harmless when swallowed but regularly produce infection when injected into the tissues of a susceptible animal. Others, such as the organisms causing typhoid fever, cholera, the dysenteries and other enteric infections, are harmless when rubbed into the abraded skin but promptly produce disease when swallowed by man.[35]

Bacteria present in a primary focal infection may spread by metastasis to set up multiple secondary foci, a condition known as *pyemia*. Other bacteria may multiply in the blood stream, a condition known as *bacteremia* or *septicemia*; the invading bacteria may become widely disseminated through the capillaries of the tissues with the production of a fulminating, *generalized infection*.

Infection is, however, in part a function of the defensive mechanisms of the host, and in some cases a bacterium capable of further spread is held in check by these defenses and sets up a *latent infection* which may flare up when resistance is reduced. Such an inconclusive outcome may result in the *carrier state*, a condition in which a virulent bacterium, fully capable

[35] Bacterial localization is discussed at greater length by Rosenow in *Newer Knowledge of Bacteriology and Immunology* (Jordan and Falk, University of Chicago Press, Chicago, 1928), p. 576

of producing disease, is harbored by a healthy and unaffected host. In this latter instance, however, the resistance of the host is not infrequently specific and a consequence of previous immunization.

Mixed and Secondary Infections.—It has long been known that an individual might be attacked by two or more infective agents at one time. Diphtheria and scarlet fever, syphilis and gonorrhea, pneumococcus pneumonia and typhoid fever, are combinations by no means unknown. It is possible that in some cases the different infections may originate nearly simultaneously, but such an occurrence is probably not common. Usually one infection precedes another, and the second is very frequently a more or less direct outcome of the first. Infection with certain microorganisms predisposes to secondary infection with the pneumococcus; acute tuberculosis may develop during an attack of measles; streptococcus invasion of the lung tissues is not uncommon in pulmonary tuberculosis. The secondary invader is commonly present in the host, but seems incapable of initiating an infection until the host defenses are weakened by the primary disease. Certain microorganisms that can cause primary infection are also frequently found as secondary invaders; pneumococci and streptococci are preeminent in this respect, and show a remarkable capacity for invading the body in the wake of other microorganisms.

Mixed infections of a somewhat different sort are those in which the principal pathogenic organism is accompanied by auxiliary bacteria, or, as some French bacteriologists have called them, *accomplices*, which by their presence influence the virulence of the chief infectious agent without themselves taking any very active part in the infectious process. Such cooperation is, of course, an instance of bacterial synergism which has been discussed previously. The aerobic bacilli which usually enter a wound along with tetanus bacilli probably facilitate the growth of the latter by providing anaerobic conditions. In other cases of mixed infections the invading bacteria may not influence one another directly but only indirectly in a joint breakdown of the defenses of the host; such is presumably the case in mixed infections of diphtheria bacilli and streptococci, and there is reason to believe that such a mixed infection is more severe than an infection with diphtheria bacilli alone—possibly spreading factor formed by the streptococci increases the permeability of the tissues to the toxin formed by the diphtheria bacilli.

RESISTANCE

It is already apparent from the foregoing considerations of bacterial virulence that, although the ability of a microorganism to produce disease is conditioned by a series of mechanisms originating with the bacterium, pathogenicity must be evaluated in terms of the resistance of the host. As a rule, a pathogenic bacterium is limited to a small number of hosts; bacteria pathogenic for animals are not ordinarily pathogenic for plants; very few of the bacteria that can infect mammals are also pathogenic for cold-blooded animals; some are even restricted to the tissues of a single species. Resistance, like virulence, is made up of many factors, some of which are known either in more or less specific form or in the terms of

generalities that serve as a cloak for ignorance; others are, in all probability, as yet unsuspected. Resistance to infection is, in a sense, somewhat more complex than virulence for, as will appear, not only are there specific barriers to infection variable with respect to species and even from one tissue to another in the body of a single animal, but the efficiency of these barriers is also a manifestation of general physiological well-being and hence they are subject to extrinsic or environmental influences.

The differentiation of resistance from natural immunity (p. 295) cannot be made with precision, for the two merge with one another and with acquired immunity without sharp lines of demarcation. In a general way they may be separated on the basis of specificity, resistance being non-specific with respect to the invading microorganism while immunity is sharply specific for a given parasite. The non-specific cellular response (p. 291) is intermediate between the two since the specific cellular immunity of the immunized animal is qualitatively the same, differing only in that it is markedly accentuated.

As in the case of virulence, the better known components of resistance are best considered one by one.

Species, Racial and Inherited Resistance.—Species of higher organisms differ greatly from one another in their resistance to any given disease, a fact that has been suggested earlier in connection with the experimental reproduction of disease. In many cases resistance to infection is relative, for disease may sometimes be produced by the administration of massive doses of bacteria to a resistant animal, but in others it appears to be absolute. Man is, for example, apparently completely immune to cattle plague, and many of the lower animals are equally resistant to some diseases of man. In general the factors underlying differences in species resistance are unknown, but in a few cases body temperature or differences in anatomical structure have been found to account for the observed variation. Pasteur's classic experiment in which he rendered the naturally resistant hen susceptible to anthrax by chilling it in cold water, and the converse of this experiment, the production of anthrax in the resistant frog by raising its body temperature to 25° to 35° C., may be explained in terms of unfavorable body temperatures. Similarly, cold-blooded animals are not susceptible to human tuberculosis and warm-blooded animals are not infected by the tubercle bacilli of cold-blooded animals. The insusceptibility of experimental animals such as guinea pigs and rabbits to the enterotoxin produced by some bacteria is possibly attributable to their lack of a vomiting mechanism. As indicated earlier, resistance to a given infectious agent is not necessarily associated with phylogenetic relationships, and there is no pattern from which the resistance or susceptibility of an animal can be predicted by logical processes; the tabulation of animals susceptible to a given disease represents information acquired largely by trial and error.

Domestic and Experimental Animals.—Not only do species of higher organisms differ in their resistance to infectious disease but the races comprising a susceptible species likewise appear to differ among themselves. There are many instances of differences in resistance to infectious disease

in varieties or strains of animals. The relative resistance of Algerian sheep to anthrax is well known and inbred Berkshire swine have been found to be highly resistant to brucellosis.[36] That this is a true racial immunity which is, as might be expected, inheritable, has been demonstrated by extensive experimental investigations with laboratory animals. The earlier studies of Wright and Lewis[37] showed that marked differences in susceptibility to tuberculosis existed between inbred families of guinea pigs, differences which were transmitted to the offspring. Later work, summarized by Webster,[38] Hill[39] and Greenwood *et al.*,[40] has been confined, for the most part, to studies on the susceptibility of mice to infection with *Salmonella typhi-murium* and similar bacteria; it has shown that resistance to such infections may be raised or lowered by selective breeding, sometimes to a remarkable degree. Similarly, strains of mice differ in their susceptibility to murine typhus,[41] and Lurie[42] has bred strains of rabbits resistant and susceptible to infection with tubercle bacilli. It is of interest that resistance to bacterial endotoxin may also be raised or lowered by selective breeding.[43] Resistance does not behave as a simple Mendelian character but is to some extent specific in that a race having increased resistance to infection with one microorganism is not necessarily unusually resistant to another.

Considerable interest has attached to the mechanisms underlying inherited resistance and susceptibility, and in recent years suggestive results have been reported by a number of workers. Thus, in Lurie's[42] studies resistance was associated with low skin permeability as assayed by intradermal inoculation of india ink, increased rate and intensity of antibody (agglutinin) response, and the development of a high degree of hypersensitivity (see also p. 305). Gowen and Calhoun[44] have shown that in mice there is a marked correlation between numbers of leucocytes and resistance to mouse typhoid. Similarly, Severins, Roberts and Card[45] have found that resistance and susceptibility of breeds of chickens to *Salmonella pullorum* infection is associated with numbers of lymphocytes.

Races of Man.—The relative resistance of races of man to infection has been the subject of considerable interest and such investigation as has been possible. Under ordinary circumstances in this country the non-white races are much more susceptible to infectious disease than the white race. There are, however, certain exceptions. Thus, the influenza epidemic of 1918 appears to have had a greater impact upon the death rates for white youths than upon that for the non-white population in the same age group.[46] Similar exception may be noted in the case of Baltimore Negroes

[36] Cameron, Gregory and Hughes: Amer. Jour. Vet. Res., 1943, *4*:387.
[37] Wright and Lewis: Amer. Naturalist, 1921, *55*:20.
[38] Webster: Medicine, 1932, *11*:321.
[39] Hill: Medical Research Council, Special Report Series, No. 196, 1934.
[40] Greenwood, Hill, Topley and Wilson: Medical Research Council, Special Report Series No. 209, 1936.
[41] Moragues and Pinkerton: Jour. Exp. Med., 1944, *79*:35.
[42] Lurie: Amer. Rev. Tub., Suppl. 1941, *44*: 125 pp.
[43] Hill, Hatswell and Topley: Jour. Hyg., 1940, *40*:538.
[44] Gowen and Calhoun: Jour. Inf. Dis., 1943, *73*:40.
[45] Severins, Roberts and Card: Jour. Inf. Dis., 1944, *75*:33.
[46] Bureau of the Census, Spec. Repts., 1943, *16*:291.

who showed a lower ratio of clinical diphtheria to immunizing infections than corresponding white children.[47]

Special interest has attached to the white and non-white tuberculosis death rates, both crude and age-specific (p. 587). Whether the observed high mortality in the non-white represents a racial susceptibility or is entirely a reflection of economic status has been the subject of considerable discussion. Studies on this question made in the Army (p. 588) would seem to indicate a true racial difference.

There also appear to be differences between the less well-defined "races" of man. There is evidence that the Irish are less resistant to tuberculosis than certain other elements of the American population, such as the Italians.[48] On the other hand, the Jewish race is considered by many to be relatively resistant to tuberculosis; in spite of a high incidence of infection, the mortality is very low.[49] To what degree the evidence supports the hypothesis that races[50] of man differ in their susceptibility to this and other diseases, such as pneumonia, is problematical, for adequate control of the environmental factors is difficult if not impossible. However this may be, twin studies on tuberculosis indicate the operation of genetic factors in the resistance of man to this disease,[51] and the question is rather one of the occurrence of practically significant genetic segregation.

A given disease, however, may be relatively mild in its effect on races of man which have been in contact with it over a long period of time, but assume a highly virulent form in other races to which it is new. Measles, for example, a mild disease to civilized man, has been a scourge to certain primitive races. In other cases, diseases originally highly virulent have become apparently less so with the passage of time; leprosy is not as widespread as it was in biblical times, and syphilis is a considerably milder disease today than it was in the sixteenth century. Phenomena such as these have been taken by some to indicate the development of a racial immunity through a selection of more resistant individuals, and by others to suggest an adaptation of the microorganism accompanied by a loss of virulence. At the present time, it is not possible to differentiate sharply between these two; possibly both effects are operative.

It should be pointed out in this connection that what might be called a pseudo-racial immunity may be manifested by a race in close association with a given infective agent. Many individuals have the disease, the survivors are immune, and this immunity is passively transferred to the offspring (see p. 300), who are infected before this passive immunity entirely disappears and consequently have the disease in a mild form but become solidly immune. The immunity is passively transferred to the third generation, and the process continues *ad infinitum* as long as the race is in contact with the disease. Some such mechanism as this appears to account for the

[47] Frost: Jour. Prev. Med., 1928, *2*:325.

[48] Guilfoy: Quart. Publ. Amer. Stat. Assn., 1907, *10*:515; Dublin: Amer. Econ. Rev., 1916, vol. 6, no. 3; Dublin and Baker: Quart. Publ. Amer. Stat. Assn., 1920.

[49] For example, see Drolet: Amer. Rev. Tuberc., 1924, *10*:280.

[50] In a number of these studies racial stocks were determined for first generation immigrants on the basis of parental birth place.

[51] *Cf.* Kallman and Reisner: Amer. Rev. Tub., 1943, *47*:549.

apparent racial immunity of the West African Negro to yellow fever; the immunity of adults arises as a result of mild infection in childhood.

Age.—The effect of the age of an animal on its resistance to infectious disease is variable, being clearly apparent in some instances and indistinguishable in others. In many cases there is a direct relation between age and resistance, the young being more susceptible than the older individuals. The suitability of the developing chick embryo as a menstruum for the cultivation of certain filterable viruses to which the chicken is apparently completely insusceptible, and the results of the experiments of Woolpert and his colleagues[52] on the infection of the guinea pig fetus, may be regarded as examples of an exaggerated susceptibility of the immature organism. The marked susceptibility of the guinea pig fetus to tuberculosis drops off after birth, and young, mature and old animals have been found[53] to be progressively more resistant to this infection. Similar results have been observed with other infections such as equine encephalomyelitis in mice,[54] St. Louis encephalitis in mice,[55] and in the resistance of the rat to diphtheria bacilli and diphtheria toxin.[56]

It is well known that the very young child does not respond well to active immunization; Sauer,[57] for example, has reported poor results in active immunization against whooping cough, and antidiphtheria immunization of the newborn is not successful. This inadequate immune response is undoubtedly at least partially responsible for infection of the very young with bacteria ordinarily regarded as harmless saprophytes. Cass,[58] for example, has reported epidemic infection of the newborn (in a maternity ward) with *Bacterium aerogenes* which took a septicemic form, and the *Bacterium coli* infection of foals known as scours is well known. Complement deficiency in the newborn[59] and less active phagocytosis by Kupffer cells[60] are possibly associated with this failure to respond to antigenic stimuli and bacterial invasion.

The rise in resistance coincident with the development of an animal to maturity has been interpreted by some workers as indicative of a "maturation immunity" in which the ability to withstand infection is associated with the ductless gland secretions.[61] Experimental studies in which precocity has been induced by the injection of androgens have, however, been disappointing, and maturation immunity must be regarded as a hypothesis which is suggested by certain clinical results but for which there is no sound experimental proof.

[52] *Cf.* Woolpert: Amer. Jour. Path., 1936, *12*:141; Woolpert *et al.*: Jour. Exp. Med., 1938, *68*:313; Gallagher and Woolpert: Jour. Exp. Med., 1940, *72*:99; Dettwiler, Hudson and Woolpert: Jour. Exp. Med., 1940, *72*:623.

[53] Smithburn: Amer. Rev. Tuberc., 1939, *39*:383.

[54] Morgan: Jour. Bact., 1940, *39*:43.

[55] O'Leary, Smith and Reames: Jour. Exp. Med., 1942, *75*:233.

[56] Seligmann: Proc. Soc. Exp. Biol. Med., 1941, *47*:241.

[57] Sauer: Amer. Jour. Path., 1941, *17*:719.

[58] Cass: Lancet, 1941, *i*:346.

[59] Traub: Jour. Path. Bact., 1943, *55*:447.

[60] Culbertson: Arch. Path., 1939, *27*:212.

[61] Hahn in Kolle, Kraus and Uhlenhuth: *Handbuch der pathogenen Mikroorganismen* Jena. 1929. Vol. 1, p. 663.

The well-known increased susceptibility of the aged to certain of the infectious diseases is reflected not only in the incidence but also in the mortality of infections such as pneumonia. The quantitative (exponential) relation between the probability of death from a given disease and age was observed many years ago (1825) and is known as Gompertz's law. The physiological alterations underlying senile debility and mortality are probably in the nature of an accumulation of degenerative changes.[62]

In practice, the relation of age to resistance to infection is complicated by many factors. The human baby, for example, is relatively resistant to a number of infections for the first six months of life as a result of passive transfer of antibodies from the mother either via the placental circulation

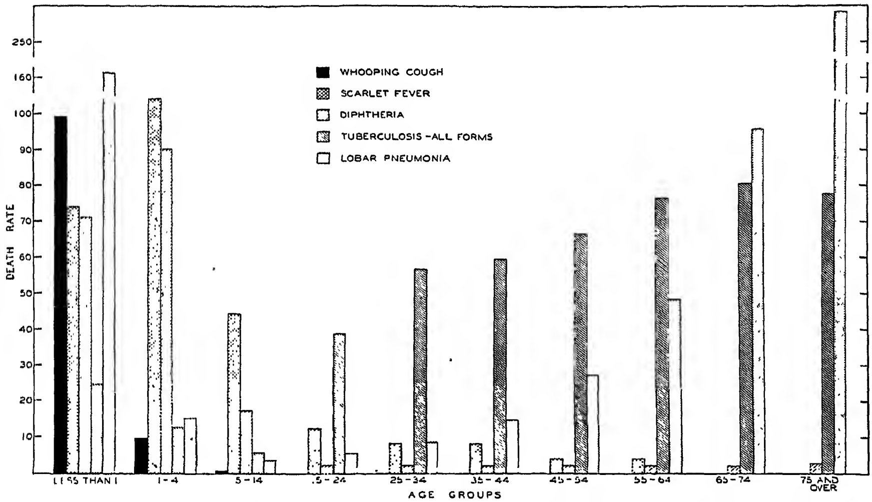


Fig. 24.—The age incidence of certain infectious diseases. Note the predominance of the diseases of childhood in the early years, the increase in tuberculosis in the young adult years and the marked increase in lobar pneumonia with advancing age. Scarlet fever rate multiplied by a factor of 40, and diphtheria rate by a factor of 10 for comparative purposes. Data for 1940 from the reports of the Bureau of the Census.

in utero or by means of colostrum after birth. Similarly, the increased resistance to a disease such as diphtheria which appears with physical development arises primarily as a result of inapparent infection rather than maturity alone. (The so-called natural antibodies are discussed on p. 296.)

Sex.—A number of diseases exhibit a difference in sex incidence; pneumonia and epidemic meningitis, for example, are more common in males than females, while scarlet fever, typhoid fever and others occur somewhat more frequently in females. Except for a few years in the forty-one-year period 1900–1940, the female death rate for white youths has been consistently lower than that for males, though the reverse has been true for

[62] Simms: Science, 1940, *91*:7.

the non-white population in this country. In the decade 1931–1940 the gap between the female and male in the non-white population has been closed and in 1940 the female and male death rates for non-white youths were exactly the same at 5.0 per 1000. Corresponding rates for the white population were 1.4 for females and 2.0 for males. In the case of tuberculosis, variability in the age-specific mortality rates indicates a differential response on the part of the sexes (p. 587). A sex differential is also indicated by the aftermath of the 1918 influenza epidemic, which appears to have had a more unfavorable effect upon female mortality than that of males in the 15 to 24 age group, as indicated by the relatively high female death rate for all racial groups for several years after the epidemic.

It has been suggested that this difference between the sexes might result from resistance having some of the attributes of a sex-linked character, or, since differences in sex incidence are most pronounced during and after puberty, something in the nature of maturation immunity may be involved. There is no sound evidence to support the first of these hypotheses. Regarding the second a certain amount of experimental evidence has been reported, the most precise relating to skin permeability, which has been found to be greater in female rabbits than in males.[63] Any explanation of the observed sex differences must, of course, take into consideration other factors such as occupation, risk of exposure and the like.

Climate and Season.—That both climate and the season of the year exert marked effects on the incidence and mortality of a number of infectious diseases is well known. In tropical climates, for example, the acute upper respiratory infections are not so common as in the temperate climates, but the dysenteries are more common in the tropics than in the temperate zones. The seasonal incidence of the infectious diseases is also common knowledge; meningococcus meningitis occurs predominantly in the winter and spring, poliomyelitis in the late summer and early fall, and so on.

In a great many instances the influence of climate and season upon the incidence of disease may be attributed to opportunities for transmission of the causative organism, such as crowding in poorly ventilated rooms, seasonal or geographic occurrence of an insect vector and so on, but in others the factors responsible for seasonal incidence are unknown. In recent years specific evidence has begun to accumulate which substantiates the opinion that resistance varies with season, temperature and similar factors. For example, the intensity of the brain reaction of mice inoculated with St. Louis encephalitis virus and that of guinea pigs inoculated with endemic typhus virus has been found[64] to have been the greatest in summer and the least in the winter over a period of years; mice adapted to existence in a moist heat have been found only one quarter as resistant to infection with hemolytic streptococci as those adapted to a cool environment;[65] the morbidity and mortality rates in murine typhus infection in mice are affected

[63] Lurie and Zappasodi: Proc. Soc. Exp. Biol. Med., 1939, *42*:741; *ibid.*, Arch. Path., 1942, *34*:151.

[64] Lillie. Dyer, Armstrong and Pasternack: Pub. Health Rep., 1937, *52*:1805.

[65] Colvin and Mills: Science, 1939, *90*:275.

by environmental temperature;[66] and the resistance of mice to pneumococcus infection is similarly affected.[67] It is not improbable that future investigation will yield systematic knowledge of the effect of climatic factors on susceptibility to disease.[68]

General Physiological Well-Being.—Whatever resistance to disease an organism may possess by virtue of species, race and the like is profoundly influenced by its general physiological state. In general, resistance is at its height when the organism is functioning normally in every respect, and is reduced by a variety of factors which interfere with and alter the normal physiological state. In some cases previous infection may reduce resistance to such a point that infection with the less virulent bacteria may take place, as in the secondary infections; in others, functional disorders such as diabetes mellitus bring about a reduction in resistance to infection. More common, however, are the deleterious effects of inadequate diet and fatigue.

Nutrition.—The relation between susceptibility to infection and faulty nutrition has been of considerable interest since the discovery of the vitamins and consequent study of the deficiency diseases, in some of which, such as xerophthalmia, infection plays a part. Attention has been directed particularly toward vitamins A (carotene) and C (ascorbic acid), and there is no doubt that a deficiency of the first of these substances results in a marked lowering of resistance. Diets qualitatively and quantitatively inadequate, not only with respect to other vitamins but in other ways also, may likewise predispose to bacterial infection.[69] Recently Cannon has emphasized the importance of adequate protein intake and he and his coworkers have shown[70] that depletion of the protein reserves of experimental animals by maintenance on low protein diets interferes markedly with antibody formation, *i.e.*, the synthesis of immune globulin. Possibly the reduced functional activity of the blood phagocytes of animals fed protein-deficient diets[71] is a related phenomenon.

Other than this, just how malnutrition brings about a reduction in resistance is not known; present information indicates that the effect is a general one. It is of some interest that this unfavorable effect is not limited to the undernourished individual but may be transmitted to offspring. Experimental studies have shown,[72] for example, that the diet of mice, genetically homogeneous in resistance to an infection, affects the resistance of offspring to a greater degree than the diet of the offspring themselves. In this connection, statistical studies[73] have suggested that the mortality rates of adult human life are in part a function of nutritive and other

[66] Moragues and Pinkerton: Jour. Exp. Med., 1944, *79*:41.

[67] Mills and Schmidt: Amer. Jour. Trop. Med., 1942, *22*:655.

[68] *Cf.* Mills: *Medical Climatology*. Charles C Thomas. Springfield, Ill. 1939.

[69] See the reviews of Robertson: Medicine, 1934, *13*:123; Clausen: Physiol. Rev., 1934, *14*:309; Jour. Amer. Med. Assn., 1935, *104*:793; Cowell *et al.*: Proc. Roy. Soc. Med., 1937, *30*:1039.

[70] Cannon, Chase and Wissler: Jour. Immunol., 1943, *47*:133.

[71] Mills and Cottingham: Jour. Immunol., 1943, *47*:503.

[72] *Cf.* Church: Amer. Jour. Pub. Health, 1939, *29*:215.

[73] *Cf.* Greenwood: Jour. Roy. Stat. Soc., 1936, *99*:674.

elements of the environment of childhood; in other words, the effect may be a delayed one, but the evidence on this point is not altogether indubitable.[74]

The marked reductions in resistance associated with inadequate diets are in no sense specific; resistance to infection in general is reduced, and there is no relation between lack of a single dietary factor and susceptibility to a particular infection. A number of attempts have been made to demonstrate such a relation; ascorbic acid, for instance, has some small capacity to neutralize diphtheria toxin, but vitamin C deficiency does not predispose to infection with the diphtheria bacillus any more than with other organisms, such as the tubercle bacillus.

Since inadequate diet, and vitamin A deficiency in particular, reduces resistance to infection, it has been supposed that resistance might be raised in hypervitaminosis. Experimental evidence, however, does not support this supposition; the normal level of resistance maintained by the organism on an adequate diet cannot be appreciably raised by the administration of vitamin A in quantities in excess of that normally required.

Fatigue.—It has long been known to the clinician that bodily rest is a valuable adjunct to the treatment of disease, and there is clinical evidence which suggests that resistance to the initial infection may be reduced by excessive fatigue. Experimental evidence on this point is scanty and to some degree conflicting, but it is probable that the unfavorable effect of fatigue on normal physiological well-being is reflected to some extent in an increase in susceptibility to infection. The normal white rat, for example, is highly resistant to anthrax, but when exhausted by work in a treadmill becomes susceptible;[75] latent *Salmonella enteritidis* infections in the same experimental animal may be activated by fatigue to such a degree that the outcome is fatal.[76] Similarly, studies on human beings have indicated that an individual may be rendered transiently susceptible to the common cold by fatigue.[77]

Other mechanisms operative in the resistance associated with general physiological well-being are obscure. The recent studies of Locke[78] have indicated that the capacity to maintain effective circulation and the ability to withstand the effects of sudden temperature changes are associated with resistance to experimental infection. The adverse effects of sudden changes in temperature and humidity on the organism, reflected in changes in the nasal mucosa, are, perhaps, a manifestation of temperature shock. Attempts to associate such shock, vitamin deficiencies, fatigue and other elements of the non-specific resistance of health with specific defense mechanisms such as the capacity to form antibodies, have not been uniformly successful.

The External Defenses of the Organism.—The cellular organization of the animal body is a closed system with respect to the outside environment from which it is separated by the skin, mucous membranes and intestinal mucosa. These structures, generally impermeable to particulate

[74] Cheeseman: Human Biol., 1938, *10*:537.
[75] Charrin and Roger: Arch. Physiol. Norm. Path., 1890, *22*:273.
[76] Boycott and Price-Jones: Jour. Path. and Bact., 1926, *29*:87.
[77] Locke: Jour. Immunol., 1939, *36*:365.
[78] *Ibid.*, 1939, *36*:159, 172, 183, 365.

material of the size of bacteria, constitute the first line of defense against invading microorganisms and one that is, for the most part, highly effective. While mechanical obstruction contributes in no small part to the efficacy of these barriers, both skin and mucous membranes also play an active part in the protection of the organism against bacterial invasion, as will appear.

The Skin.—As a rule, the unbroken skin presents a more or less impassable barrier to microorganisms. Bacteria are found normally on the skin between the superficial horny cells, but ordinarily are not able to penetrate deep into the tissues unless favored by some cutaneous injury, such as a wound or burn. The ducts of the sweat glands and the hair follicles are, however, vulnerable points, and experiment has shown that it is possible for bacteria to penetrate the skin through these channels.

The skin, however, is not an inert surface upon which bacteria may survive, but is, as experimental work of the last few years has shown, actively bactericidal. Arnold and his co-workers[79] have found that bacteria which do not occur normally on the skin, such as *Bacterium prodigiosum*, the typhoid, colon and enteritidis bacilli, and hemolytic streptococci, are rapidly (in some cases within ten minutes) destroyed when swabbed on the clean skin of the palm of the hand. The autosterilizing capacity of the skin is markedly reduced by dirt and is apparently a property of live skin; the skin of a cadaver dead only fifteen minutes showed little or no bactericidal activity. Bacteria normally present upon the skin, such as the white staphylococci, are not appreciably reduced in numbers when swabbed on the clean skin, a fact which probably accounts for their constant presence on the body.

The Conjunctivae.—Bacteria and dust particles settling in the eyes are removed relatively rapidly by the mechanical flushing effect of the tears. The lacrimal secretions contain a substance, *lysozyme*, which is also present in certain tissue extracts and in egg white, which destroys some species of saprophytic bacteria. Fleming[80] who discovered lysozyme, described one species of bacteria, *Micrococcus lysodeikticus*, which is particularly susceptible to the action of this lytic substance; as high a dilution as 1 : 40,000 of tears has brought about complete lysis of these microorganisms. None of the pathogenic forms, however, appears to be unusually susceptible to the action of lysozyme, and it probably plays no important part in the protection of the organism against invasion.

The Nose, Nasopharynx and Respiratory Tract.—Bacteria and other particulate material present in inspired air are rapidly removed by passage through the tortuous nasal passages lined with mucous membrane to whose moist surface they cling. In this way air is largely freed from bacteria in the upper respiratory passages, those that pass the larynx are caught in the bronchi and few reach the ultimate ramifications of the bronchioles.

[79] *Cf.* Arnold, Gustafson, Montgomery, Hull and Singer: Amer. Jour. Hyg., 1930, *11:*345.

[80] Fleming: Proc. Roy. Soc., 1922, Series B, *93:*306; Lancet, 1929, *i:*217; Proc. Roy. Soc. Med., 1932, *24:* (Sec. Path.) 1. See review by Mesrobeanu and Noeppel: Arch. roumaines path. exptl. microbiol., 1938, *11:*247; and by Thompson: Arch. Path., 1940, *30:*1096.

The process is so efficient that expired air contains almost no bacteria except those that are expelled in droplets by sneezing, coughing and talking.

The moist film which covers the mucosa of the upper respiratory tract and in which bacteria removed from inspired air and those arriving via the lacrimal secretions are embedded consists of mucus, a thin, highly viscid substance that, in a sense, constitutes a continuous web or membrane overlying the surfaces within the nose, sinuses, pharynx and esophagus. This film of mucus is in constant motion as a result of the activity of cilia which sweep the mucus and its bacterial content toward the oropharynx, where it is swallowed. The exchange of mucus is rapid, that covering the posterior two thirds of the nose is replaced every ten or fifteen minutes, while that over the anterior third is removed every hour or two.[81] Although mucus itself has no bactericidal activity, when combined with ciliary activity it constitutes a remarkably efficient means of ridding the upper respiratory passages of bacteria.

Lysozyme is, of course, present in the nasal mucus and it has been observed[82] recently that the normal serous secretion of the nose contains a virus inactivating agent (VIA) distinct from lysozyme. The activity is virucidal for the influenza and certain other viruses inactivated by sodium desoxycholate but what part it plays in resistance to infection is not clear.

The bacteria that penetrate the upper respiratory passages and lodge in the bronchi and bronchioles are probably phagocytosed by the fixed alveolar epithelial cells and the wandering leucocytes that enter the bronchioles and sacs, a process discussed at greater length elsewhere.

The Mouth, Stomach and Intestinal Tract.—The healthy mouth ordinarily contains great numbers of bacteria, but, except in the case of those organisms which have established themselves, this flora is a transient one, the microorganisms being constantly removed through the flushing action of saliva and as constantly supplemented by fresh contamination. Saliva is but mildly bactericidal,[83] and the removal of bacteria is practically entirely a mechanical process.[84] The microorganisms flushed to the back of the mouth meet with those from the nose and, with them, are swallowed.

Bacteria reaching the stomach are subject to the strongly acid environment of the normal gastric juice, and there is no doubt that the great majority of them are destroyed there. Some do, however, reach the intestinal tract, perhaps because they are embedded in solid particles of food and thus protected or because they are able to withstand a short exposure to the bactericidal action of the gastric secretions. Generally, very few viable bacteria are found in the stomach, but the numbers of microorganisms increase in the small intestine with the rise in *p*H from the duodenum to the ileum. The large intestine contains great numbers of bacteria derived not only from the upper levels of the intestinal tract but also from

[81] *Cf.* Hilding: Ann. Int. Med., 1932, *6:*227.

[82] Burnet, Lush and Jackson: Brit. Jour. Exp. Path., 1939, *20:*377; Francis: Science, 1940, *91:*198.

[83] *Cf.* Van Kesteren, Bibby and Berry: Jour. Bact., 1942, *43:*573.

[84] Bloomfield: Amer. Rev. Tuberc., 1922, *5:*903; Johns Hopkins Hosp. Bull., 1922, *33:*61, 145.

the multiplication of bacteria present in the intestines as normal inhabitants. As in the respiratory passages, mucus plays an important part in the mechanical removal of bacteria. Here, however, the mucus does not form a uniform coating over the intestinal mucosa but is present as a meshwork. The villi free themselves from particles by movements which bring them in contact with the mucus to which the particles, including bacteria, adhere. The mucus, with the embedded microorganisms, is rolled up into small masses and moved outward by the peristaltic movements of the bowel.[85] Bacteria, then, which enter the mouth and upper respiratory tract are eventually extruded with the feces.

The Genital Tract.—The normal genital tract is remarkably free from bacteria. The urethra in both male and female is normally sterile, a consequence, perhaps, of the flushing action of the slightly acid urine. The few bacteria that may be present are confined to the region of the meatus. The normal vaginal secretion is acid and is markedly bactericidal toward most species of bacteria.

Normal Flora.—A microorganism somewhat better able to resist the defensive mechanisms of the host but at the same time unable, except when resistance is reduced to a low level, to invade the body tissues, may exist in conjunction with the host as a part of the latter's "normal flora." The staphylococci which are able to resist the bactericidal action of the skin are almost invariably present on these surfaces and are regarded as "normal" inhabitants. The scanty bacterial flora of the vagina, on the other hand, is composed almost entirely of aciduric bacteria; and the bacterial types present in the intestines are determined to a considerable extent by the type of food material present, *i.e.*, the diet of the host, and by the *p*H of the various intestinal levels. Similarly, certain kinds of bacteria, such as lactobacilli, spirochetes, various cocci and the like, exist in the mouth in the interstices between the teeth and in and under tooth plaques and constitute a normal flora characteristic of this region. The bacteria commonly present in the nose and throat consist of still different forms, such as pneumococci, Friedländer's bacillus, green and hemolytic streptococci, etc. These organisms, while in a sense a normal flora, are not so well established as the flora of some other regions, and the nature of the microorganisms present may be determined in large part by the kind of bacteria which are constantly entering the upper respiratory tract.

As long as the resistance of the host is maintained at a sufficiently high level, the bacteria constituting the normal flora do no harm. If, however, resistance is reduced in some manner, the more virulent forms may invade the tissues and set up an infection. The congestion of the nasal mucosa, and the consequent interference with ciliary activity and the movement of mucus, which follows the temperature shock of chilling, not infrequently makes possible infection by bacteria such as hemolytic streptococci or pneumococci, which are already present.

[85] Florey: Jour. Path. and Bact., 1933, *37*:283.

CHAPTER 9

THE TRANSMISSION OF INFECTION

Whatever the pathogenic powers of a bacterium and the efficiency of the defensive mechanisms of the host, an essential preliminary to the production of infectious disease is a meeting of the parasite and its prospective host. In some instances in which the bacterium is naturally saprophytic, it enters the body by accident, so to speak; such, for example, appears to be the case with tetanus, gas gangrene and similar infections. In most instances, however, the bacteria that produce disease are more or less closely adapted to a parasitic existence, and pass from one animal body to another with only a relatively brief sojourn in the external world. In general, then, the transmission of infection is a process in which the causative microorganism is transferred, either directly or indirectly, from a diseased to a healthy susceptible animal.

The elucidation of the mechanisms involved in this transfer is a matter of considerable practical as well as theoretical importance. If the sequence of events that precedes infection is known, it may be, and often is, possible to interrupt it at its most vulnerable point and thereby control the spread of disease. From the theoretical point of view, disease is by no means entirely a matter of host resistance and microbic virulence; it is, in a very real sense, the outcome of the interaction of the host and parasite populations. It is at this point that the study of the infectious diseases transcends the bacteriology of clinical medicine with its emphasis on the individual case, and assumes broad biological significance as a problem in interspecies competition.

The equilibrium that tends to become established between the host and parasite populations is an unstable one in that the factors which determine it—*i.e.*, the character of the host population in particular and possibly that of the parasite population, as well as the environmental factors which affect their relationship—are constantly shifting, and the equilibrium ever has a tendency to establish itself at a new level. The shift may be a sudden and violent one whose outward manifestation is an explosive outbreak of disease or, less commonly, may take the form of a gradual increase or decrease in the incidence of the disease.

The factors associated with the maintenance or shift of this equilibrium are the subject matter of *epidemiology*.[1] The term epidemiology is best regarded in this broad sense and therefore includes the study of the transmission of *endemic* disease, *i.e.*, disease which has a low incidence but is

[1] For a general discussion of the principles and methods of epidemiology see Frost: *Collected Papers*, pp. 493–542. Commonwealth Fund. New York. 1941. A brief discussion of theoretical epidemiology is given by Aycock and Russell: Amer. Jour. Med. Sci., 1943, *206*:399.

constantly present in a population, as well as the study of *epidemic* disease, *i.e.*, disease of high morbidity which is only irregularly present in clinically recognizable form. In this connection it may be noted that the term *pandemic* is often applied to an epidemic of unusually great proportions. These categories, although useful, are not mutually exclusive; a disease endemic in a community may, at times, attain the proportions of an epidemic and later subside to an endemic level.

Essential to knowledge of the epidemiology of disease are certain characteristics of the etiologic agent and of the clinical infection which determine the possible channels of transmission. The more important of these are:

(1) the route by which the infective agent enters the body;
(2) the route by which the infective agent leaves the body;
(3) the resistance of the microorganism to the deleterious effects of the outside environment;
(4) presence or absence of an intermediate host; and
(5) the relation between frank, clinically recognizable disease and the discharge of virulent bacteria from the body.

The Carrier.—With regard to the last, additional explanation is desirable. In the early days of bacteriology it was assumed that the contact of host and parasite could have only one or the other of two outcomes; either no infection occurred, owing presumably to high resistance on the part of the host, or clinically characteristic disease developed in the individual. More recently it has become clear that an intermediate state, the establishing of a symptomless infection, may occur. Such infections are, of course, inapparent and concealed and may be demonstrated only by isolation and identification of the infectious agent. An individual so infected is termed a carrier.

Two types of carriers are commonly differentiated, the casual carrier who harbors the microorganism temporarily, a matter of a few days or weeks, and the chronic carrier who remains infected for a relatively long time, sometimes throughout life. Such individuals serve, of course, to disseminate the infectious agent. In the first group are the great majority of carriers of the diphtheria bacillus, meningococcus, pneumococcus, certain streptococci, etc., and the second includes carriers of certain of the enteric bacilli, especially the typhoid bacillus. A third type of carrier is often differentiated, the convalescent carrier who remains infected for a greater or lesser length of time after recovery from the disease. These last do not, of course, fall into the category of concealed infections. Sharp separation is sometimes not possible, however, for the casual or chronic carrier may, in fact, be convalescent from the disease in a form either atypical or so mild as to go unrecognized (ambulatory cases).

While it is now commonplace to recognize the existence of the carrier state, the implications of the general principle that infection may occur without disease are frequently neglected. Thus it follows that clinically apparent infections may constitute only a part, in some instances only a very small part, of the infections continually taking place. Clearly, then, if an important proportion of the infections are of the concealed type, a

reasonably accurate estimate of the extent to which the infection is disseminated in the host population cannot be arrived at on the basis of cases of the disease. The implications of this are several. For example, diseases which occur sporadically and do not seem to be easily or often transmitted from the sick to the well, such as poliomyelitis, meningococcus meningitis, lethargic encephalitis, pneumococcus pneumonia, etc., may be as widely disseminated and readily communicable as measles or the common cold, but the clinically distinctive disease is the exception rather than the rule. Furthermore, the rise or decline of infectious disease or its age or geographical distribution may not reflect a corresponding variability in the prevalence of the infection but may, rather, be a consequence of variation in the case-carrier ratios. Though here inferred from the observed occurrence of the carrier state, none of these possibilities remains purely hypothetical for all have been found to exist. Thus, carriers of virulent pneumococci do not occur predominantly in the higher age groups, nor diphtheria bacillus carriers in the school child, where the morbidity of these diseases is highest; diphtheria bacillus carriers are as common in the tropics as in temperate climates despite the relative rarity of clinical diphtheria in the hot climates, and so on. In many other cases, such as that of poliomyelitis, such a situation is suspected but technically difficult to prove. It will be obvious, therefore, that the recognition of the carrier state and its implications is basic to sound epidemiological thinking and of primary importance to the understanding of the mechanism of spread of the infectious diseases.

The epidemiological factors given above are most readily and most satisfactorily determined by experimental study when a disease is of known etiology, but sometimes may be approximated to a relatively satisfactory degree by indirect evidence. For example, although the famous Broad Street Pump epidemic occurred prior to the discovery of the etiology of cholera, the indirect evidence plainly indicated to Snow that the infective agent left the body in the feces and entered the gastro-intestinal tract via the contaminated well water.[2]

Epidemiological Types of Infectious Disease.—On the basis of such fundamental information, the infectious diseases may be separated into a number of *epidemiological types* which, despite certain limitations, serve to illustrate the diversity of ways in which infection may be disseminated.[3] A rough classification, based on the assumption that the human being is the recipient of infection and that the control of diseases of man is the point at issue, follows:

(1) diseases of lower animals transmissible directly to man (rabies, tularemia, glanders, etc.);
(2) diseases of animals or man transmitted by insect vectors in which
  (*a*) the insect acts as a mechanical carrier (the house fly and typhoid fever),
  (*b*) the parasite multiplies in the insect vector (bubonic plague),

[2] The original papers have been reprinted under the title *Snow on Cholera.* Commonwealth Fund, New York. 1936.

[3] Epidemiological types of infectious disease are discussed briefly by Baker: Amer. Jour. Trop. Med., 1943, *23*:559.

(*c*) the parasite is transmitted from one insect generation to the next by egg infection (spotted fever), and
(*d*) the parasite undergoes a portion of its life cycle in the insect (malaria);
(3) diseases of animals or man transmitted indirectly
(*a*) by water (the enteric infections such as typhoid fever, cholera, etc.),
(*b*) by milk (scarlet fever, bovine tuberculosis, undulant fever, etc.),
(*c*) by food (typhoid and paratyphoid fevers), and
(*d*) by inanimate objects or fomites, such as books, towels, etc. (scarlet fever, diphtheria and the like);
(4) diseases of man transmitted directly
(*a*) by infective droplets—air-borne infection (the respiratory diseases and others), and
(*b*) by direct contact (the respiratory and venereal diseases in particular).

Air-Borne Infection.—Of these modes of transmission air-borne infection is one of the most important. In recent years Wells[4] and his associates have shed new light on the mechanism of transmission of disease, especially respiratory disease, from man to man by the experimental elucidation of droplet infection. It was postulated many years ago by Pflugge that such diseases could be transmitted by infective droplets. The droplets which he studied, however, were greater than 0.1 mm. in diameter and fell to the ground soon after expulsion by sneezing or coughing, seeding the air for only negligible distances. Epidemiological considerations, however, seemed to demand air-borne infection effective at considerable distances. Wells and his co-workers have shown that Pflugge's evidence was incomplete and that under the usual conditions of humidity particles or droplets smaller than 0.1 mm. in diameter are evaporated completely before reaching the ground, leaving suspended nuclei consisting essentially of organic matter, salts and bacteria, to become, for all practical purposes, a part of the atmosphere. The survival of pathogenic microorganisms in such nuclei is, of course, largely a matter of their resistance to drying, and it has been shown that some of the respiratory pathogens, including viruses such as that of influenza, may remain viable and infective for many hours under these circumstances.[5] The process of expulsion of such droplets in coughing, sneezing and talking has been photographed by Jennison[6] and is illustrated in Fig. 25. Studies of the air in various types of rooms under various conditions have yielded data on the quantitative aspects of air contamination; green streptococci have been used as indicators of air pollution in much the same manner as *Bacterium coli* is used for studies on water pollution. In general, enormous numbers of bacteria of respiratory origin have been found in the air of crowded rooms, especially when the occupants are sneezing, and there can be no doubt of the significance of these observations in relation to the explosive spread of respiratory disease in a non-immune population.

As may be inferred from the outline above, a disease not infrequently has a certain epidemiological character which may at times be even more familiar to the epidemiologist than the causative agent is to the laboratory

[4] See the review articles by Wells: Jour. Amer. Med. Assn., 1936, *107*:1698; and by Wells and Wells: Amer. Jour. Med. Sci., 1943, *206*:11.
[5] *Cf.* the review by Buchbinder: Jour. Amer. Med. Assn., 1942, *118*:718.
[6] Jennison: Amer. Assn. Advancement Sci., Pub. No. 17, 1942, p. 106.

worker. In the case of epidemic influenza, for example, the disease is, at present, an epidemiological rather than clinical entity and is diagnosed with reasonable accuracy only during epidemic periods. Similarly, epidemi-

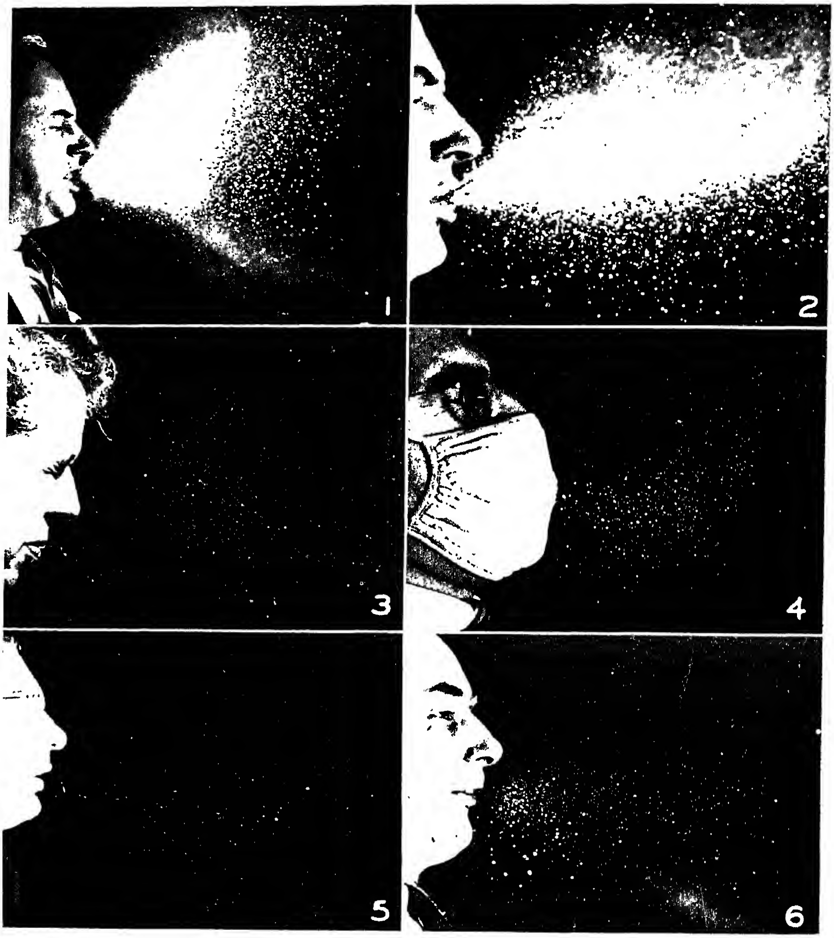


Fig. 25.—The atomization of mouth and nose secretions demonstrated by high speed photography. *1*, A violent sneeze in a normal subject; note the close approximation of the teeth, resulting in effective atomization. *2*, Head cold sneeze; note the strings of mucus and the less effective atomization of the viscous secretions. *3*, A stifled sneeze. *4*, Sneeze through a dense face mask. *5*, Cough; note the lesser discharge than in the uninhibited sneeze. *6*, Enunciation of the letter f. (Jennison.)

ological studies have shown the existence of two kinds of smallpox, the one a relatively innocuous variety with a low case fatality and the other a severe disease (so-called malignant or "black" smallpox) with a high case

fatality. The difference between these varieties is real and of no small practical importance even though indistinguishable by clinical observation of the individual case (p. 803).

The epidemiology characteristic of a given disease shows, on the one hand, certain, and sometimes close, relationships to other similar infections, and, on the other, a certain variability which arises as a result of transmission in more than one way. The enteric infections, cholera, typhoid fever and the bacillary dysenteries, are similar to one another in epidemiology but are quite different in this respect from the respiratory diseases or the insect-borne diseases. The epidemiology of typhoid fever, however, is variable within limits and depends to some degree upon whether the disease is water-borne, milk-borne or transmitted by food or contact. Clearly, then, although it is possible to speak of certain broad principles of epidemiology, the epidemiology of a disease is in itself a special case and must necessarily be considered elsewhere (see later chapters) under the head of that disease.

The epidemiological character of a disease is dependent primarily upon certain aspects of the microorganism and the clinical infection as indicated above, and secondarily upon the habits, environment and mass susceptibility of the host population. To return to the example of typhoid fever: any mode of transmission is necessarily one that provides a connecting link between infectious fecal matter and the mouth of a susceptible human being, and the possibilities are, to this extent, limited. The age, sex and seasonal incidence, to some extent the case fatality, and the geographical and sociological distribution of cases are reflections of both the mode of transmission and the character of the population. In the case of water-borne typhoid, the drinking water supplies the connecting link, and the ensuing epidemic, limited to the area supplied by the contaminated water, shows no respect for age, sex or economic status. Milk-borne typhoid, on the other hand, geographically limited by the route by which the infected milk is delivered, exhibits an increased incidence in the lower age groups and among females, and is somewhat more frequent among those of higher economic status. Other modes of transmission are similarly reflected as minor variations in the epidemiological character of the disease.

Although the characteristics of the infectious agent and the clinical disease determine the *means* by which disease may be transmitted, the *extent* of its spread is a mass phenomenon determined by the character of the host and parasite populations and, as a corollary, their interaction with one another.

The Bacterial Population.—The well-known variation in the severity and "contagiousness" of the infectious diseases is a consequence of corresponding variation from one species of pathogenic bacteria to another in their ability to invade the body tissues and, once established, to produce clinical disease. As indicated in a previous chapter, a single species is potentially variable in these respects, for such variation can be induced by appropriate experimental manipulation. The possibility of such intraspecies variation in a bacterial population existing under natural conditions is one that has intrigued students of infectious disease for many years.

It is tempting to account for the genesis and rise of epidemic disease by assuming that the causative agent of a disease of endemic proportions gains in virulence by successive passage from person to person until its pathogenicity is so enhanced that an epidemic ensues. Similarly, a sojourn in a host population containing an increasingly large proportion of immunes might be expected to result in a diminution in the virulence of the microorganism and consequent subsidence of the epidemic. Furthermore, successive epidemic waves might conceivably result from periodic fluctuations in the virulence of the parasite. Unfortunately for such an explanation, there is little or no direct evidence that alterations in virulence play an important part in the evolution of single or secondary epidemic waves, and, in nature, bacterial virulence appears to be a relatively stable character.[7]

On the other hand, differences in the severity of a single disease from one epidemic to another are, in part, attributable to the existence of strains of the infectious agent which differ from one another in virulence. Benign and malignant smallpox referred to above is a case in point, and some workers believe that some strains of the diphtheria bacillus produce a more severe disease (*i.e.*, with a higher case fatality) than others (p. 556). Although variable from one strain to another, available evidence indicates that within a single strain virulence does not fluctuate to a demonstrable degree.

Possibly attributable in part to alterations in bacterial virulence are the changes in morbidity and mortality of some diseases such as scarlet fever, syphilis and tuberculosis, over long periods of time. In the case of scarlet fever the twenty-five years prior to 1830 was a period of very low death rates and was followed by a forty-year period of high death rates. Since then the death rate has declined and, though the incidence remains high, the case fatality is relatively low. In other diseases only a decline has been observed. Syphilis is no longer the scourge it was in the sixteenth century, and the present decline in tuberculosis began before the institution of preventive and therapeutic measures. In still other diseases, such as measles, no such long-term alterations in prevalence have been observed. Information is as yet too limited to assess these phenomena; possibly in some diseases there are long-term periodic fluctuations in bacterial virulence (this may be an artifact and represent only variations in the prevalence of virulent "epidemic" strains of the parasite) while in others an adaptive reduction in virulence or increase in resistance on the part of the host or a combination of both may play a part.

In general, it may be said that, in the short view, the bacterial population, as it exists in nature, is remarkably stable in so far as its ability to produce disease in a host population is concerned. Although the severity of a disease may and often does, vary from one epidemic to another, variation in virulence is not an important factor in the single epidemic wave. Over long periods of time, however, alterations in virulence may contribute to the changes in morbidity and mortality observed in some of the infectious diseases.

[7] The case for alterations in virulence during an epidemic wave has been presented at length by Zinsser and Wilson: Jour. Prev. Med., 1932, *6*:497.

The Host Population.—In contrast to the relative stability of the bacterial population, the human population is highly variable in its resistance to infection, and the variation, attributable to both intrinsic and extrinsic factors, is not infrequently of such magnitude that its consequences are of considerable practical importance.

Since a population, human or infrahuman, is composed of individual organisms, it follows that its character is determined by the nature of these individuals and their relations to one another, and its reaction to an external influence is expressed in terms of the aggregate of the reactions of its members. The response of a human population to an infectious disease is, of necessity, measured in terms that are composites of the responses of the individual members of the population, in short, by some method of counting. Such counting is, of course, the basis of statistics, and the statistical method, with its ramifications and refinements, is a powerful tool which makes possible the study of the response of human populations to disease—a response which is measured in terms of rates, ratios, life tables and similar numerical devices.

Of the intrinsic factors which determine the response of a human population to an infectious disease, one of the most important is age distribution. The quantitative predominance of the lower age groups characterizing an immature population declines with population growth while the higher age groups correspondingly increase. As a consequence, the diseases of childhood and early adult life, such as diphtheria, tuberculosis and the like, are relatively prevalent in an immature population but become progressively less so with the passage of time, while the diseases of old age increase in incidence with the maturing of the population.[8] The frequency of an infection is expressed as a rate, either as the number of persons infected in a given unit of time or *incidence*, or as the total number of persons infected at any one time or *prevalence*. Correction of morbidity and mortality rates is a practical necessity and is made either by the use of *specific rates*, *i. e.*, the proportion of cases or deaths within a specified age group, or by the use of *standardized rates* which are not the observed rates but rather what the observed rates would be if the age distribution of the population were that of a standard or reference population.[9]

The sex distribution of a population and its racial composition are of somewhat lesser practical significance, although in some communities in the United States the high mortality rate of the Negro has necessitated the use of race specific mortality rates.[10]

[8] For a discussion of these problems see Perrott and Holland: Milbank Mem. Fund Quart., 1940, *18:*359.

[9] Two standards have been used in Europe, one, the population of Sweden as it existed in 1890, and the other, the population of England and Wales as shown by the 1901 census. The first is standard in age distribution only, while the second is standard for both age and sex. In this country the population of the entire country is usually taken as a standard, and the populations of states or other portions of the country compared or adjusted to it.

[10] For a discussion of the methods of population study with respect to disease see Pearl: *Introduction to Medical Biometry and Statistics*, 3rd ed. W. B. Saunders Company. Philadelphia. 1940.

The extrinsic factors which alter the resistance of a host population to the spread of disease may exert their effects in either or both of two ways: first, by influencing the resistance of part or all of the individuals comprising the population; and, second, by influencing the relationships between individuals. Perhaps the most important factor in the first category is active individual immunity. If a sufficient portion of a population is immune to a disease as a result of artificial inoculation or recovery from an attack, the resistance of the entire group to epidemics of that disease is of a high order, a phenomenon which has been termed *herd immunity*. Other factors may reduce the resistance of the individual; in times of stress

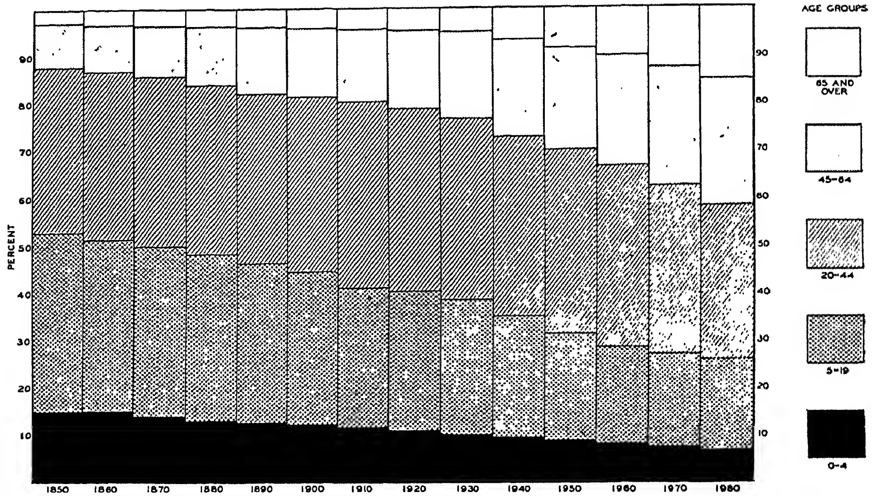


Fig. 26.—Changing age distribution in the population of the United States. Estimates 1940 to 1980 by Thompson and Whelpton.

or calamity, for example, when relatively large groups are undernourished, fatigued and exposed to inclement weather, epidemic disease may spread with great rapidity.

Equally important to the resistance of a population to epidemic disease are the factors which determine the interrelationships of its members. Crowding in large gatherings or the enforced close association arising from inadequate housing facilities obviously provides opportunity for the dissemination of respiratory and other diseases transmitted directly from man to man, and, as well, certain indirectly transmitted infections, such as louse-borne typhus fever. Similarly, the spread of enteric infections is, to a large extent, dependent upon sanitary facilities and the solution of the twin problems of water supply and sewage disposal. Group practices which support a large rat population make bubonic plague a potential menace, and the presence of large numbers of mosquitoes of the appropriate species allows the wide dissemination of malaria and yellow fever. These and other

factors, political, sociological or economic in nature, obviously exert no small influence on the resistance of a population to the spread of disease.

The Interaction of Host and Parasite Populations.—It will be clear from the foregoing discussion that the interaction of the host and parasite populations is a highly complex phenomenon. Even assuming that the parasite population, when the parasite is a pathogenic bacterium, remains relatively constant in its ability to produce disease, the resistance of the host population is in a constant state of flux and the equilibrium between the two is rarely a "steady state." As has been indicated, the relation between host and parasite populations is a part of the general problem of interspecies competition, and has been studied at length, particularly by the economic entomologist[11] and the experimental zoologist.[12] The infectious diseases of man constitute a series of special cases of the host-parasite relationship, differing from one another with respect to mode of transmission, incubation period, period of infectivity, immunity, case fatality, etc.[13] The studies on infectious disease have taken two forms: one, the theoretical analysis of epidemic spread; and the other, the experimental investigation of controlled epidemics among populations of laboratory animals, *i. e.*, experimental epidemiology.

Theoretical Analysis.—The theoretical treatment of the dissemination of infectious disease in a susceptible population is exceedingly difficult because of the tremendous number of variables which are involved. If, however, certain simplifying assumptions be made, analysis in terms of the mathematics of probability yields information of considerable significance, and a certain insight into the evolution of the single epidemic wave may be gained.

The evolution of the single epidemic wave is best considered here by the arithmetic method of Frost,[14] which involves finite differences, although other methods based upon the calculus have been developed.[15] Let C = the number of cases, S = the number of susceptibles and N = the contacts per day, then r, the contact rate per day, is given by

$$\mathrm{N} = \mathrm{rCS} \text{ or } \mathrm{r} = \frac{\mathrm{N}}{\mathrm{CS}}$$

It is assumed that each case is infectious, that one contact suffices to produce the disease in a susceptible individual, that multiple contacts are disregarded, and that the unit of time (the day) is small enough so that C and S do not change materially during that period. Then the number of contacts per unit time t (the day) is

$$\mathrm{Nt} = \mathrm{rCSt}$$

[11] See the reviews by Nicholson: Jour. Animal Ecol., 1933, *2*:132; and by Thompson: Parasitology, 1939, *31*:299.

[12] Gause: *The Struggle for Existence.* Williams & Wilkins Company. Baltimore. 1934.

[13] For a discussion of this interaction in streptococcal infection in man see Coburn and Pauli: Jour. Exp. Med., 1941, *73*:551.

[14] Frost: Cutter Lecture, 1928, unpublished. Method outlined by Zinsser and Wilson: Jour. Prev. Med., 1932, *6*:497.

[15] See Soper: Jour. Royal Statistical Soc., 1929, *92*:34.

The probability of contact is, therefore,

$$p = \frac{Nt}{S} = rCt$$

and the probability of avoiding contact is

$$q = 1 - p = 1 - rCt$$

Since there are $1/t$ units of time in the entire period, the chance of avoiding contact over this period is

$$Q = (1 - rCt)^{\frac{1}{t}} = e^{-rC}$$

Therefore, the chance of at least one contact is given by

$$P = 1 - e^{-rC}$$

and the number of new cases infected during the day is

$$PS = (1 - e^{-rC})\, S$$

Assuming the incubation period to be one day—*i. e.*, the contact of one day is the case of the next—a theoretical epidemic wave may be built up by a series of substitutions in the last equation. Starting with 10,000 susceptibles, one case, and a contact rate, *r*, of .0002, the first day,

$$(1 - e^{-.0002})\ 10{,}000 = 2 \text{ (new cases).}$$

the second day,

$$(1 - e^{-.0006})\ 9998 = 6$$

the third day,

$$(1 - e^{-.0018})\ 9992 = 18$$

and so on through the complete epidemic wave. Various modifications such as limiting the period of infectivity through the introduction of case fatality and the development of immunity, extending the incubation period, and the like may, of course, be made.

Theoretical epidemics built up in this way show a remarkable similarity to observed epidemics of disease, and, although the factors entering into the determination of the value of *r* are highly complex, it is evident that the probability of chance contact is a factor of primary importance in the evolution of the epidemic wave. In a population consisting largely or entirely of susceptibles, this probability is large and the disease spreads rapidly; but, as the number of susceptibles is reduced by conversion to cases, immunes and fatalities, the probability diminishes and the epidemic subsides.[16] It may be noted that the term "contact" is used to mean "effective contact" and more than one meeting with a case may be required to make up an effective contact. It is often said that an important factor in epidemic spread is dosage, *i.e.*, the number of microorganisms that a susceptible individual encounters within a definite period of time. An adequate "dose" is, clearly, an effective contact.

[16] For experimental studies on the mathematical theory of epidemics see Kermack and McKendrick: Jour. Hyg., 1939, *39*:291 *et ante*. Also the summary by McKendrick: Edinburgh Med. Jour., 1940, *47*:117.

The nature of herd immunity will become clear at this point, for the higher the proportion of immunes in a population the smaller the probability of effective contact between case and susceptible; *i.e.*, many of the contacts will be with immunes, and the population exhibits a group resistance to epidemic disease which may be of such a high order that an epidemic is no longer possible and the disease smoulders in an endemic form as a result of the importation of new cases or the persistence of infection in healthy carriers whose contacts will give rise to an occasional case. A susceptible member of such an immune population, then, enjoys an immunity that is not of his own making but arises as a result of his membership in the group. A measure of this protection is given as Q in the above equations.

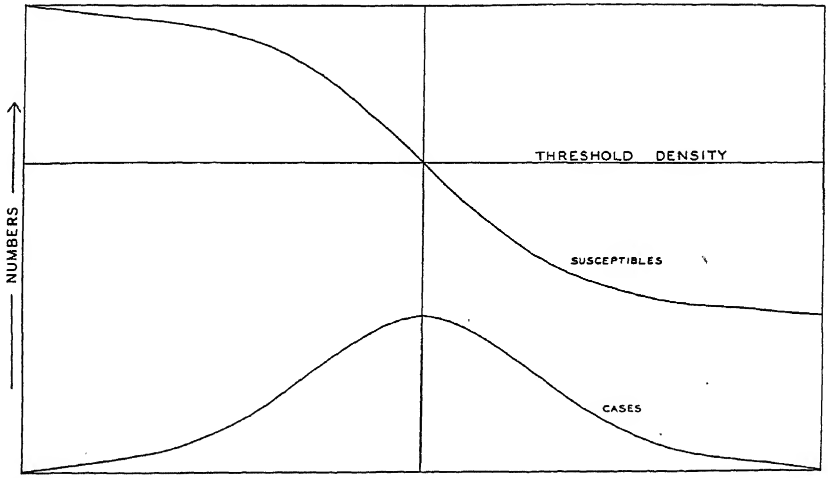


Fig. 27.—Diagrammatic representation of the course of an epidemic wave in terms of numbers of cases (lower curve) and numbers of susceptibles (upper curve). Note the coincidence of the peak of the epidemic wave with the threshold density of susceptibles. (After McKendrick.)

Clearly there will be a critical proportion of susceptibles and immunes in the population; with a greater number of susceptibles epidemic disease can develop and with a lesser number it cannot. This critical point has been termed *threshold density* and is that which prevails at the peak of the epidemic wave. The total number of cases in the epidemic will, then, be twice the number of susceptibles in excess of this threshold density present at the beginning, and following subsidence of the wave the population is left with a greater or lesser degree of herd immunity.

Such precise relationships are only approximated in nature. In practice the threshold density fluctuates and is a function in part of dosage. When the prevalence of a disease is high a given individual will be subjected to a greater number of bacteria per unit time; some, previously immune to

smaller doses, will become susceptibles under such circumstances and the "effective concentration," so to speak, of immunes declines with consequent effect on the herd immunity to epidemic disease. This effect of dosage on the host population has been termed *infection pressure* and is a matter of considerable practical importance. Successive epidemic waves may, then, occur as a consequence of increased infection pressure as well as through the accumulation of new susceptibles (see below). Recurrent waves of a disease that is a clinical but not an etiological entity are, of course, another matter.

The question of the relationship between an infectious disease and a susceptible population over a long period of time is somewhat more complex. Mathematical treatment such as the Ross malaria equations and Martini's equations for immunizing diseases[17] is too far removed from reality to have much practical significance, principally because the mathematical approach requires that the parameters remain constant over long periods of time. This condition is probably rarely if ever satisfied; it is well known, for example, that within the last century both diphtheria and scarlet fever, previously of low prevalence, took on a malignant character and higher prevalence for some decades and then within the last forty years have gradually declined in both prevalence and severity. It is of some interest, however, that the differential equations of Martini, for example, show one stable equilibrium at the origin and another at a positive value which is approached by a series of oscillations above and below the final state of equilibrium so that a series of epidemic waves appears. These solutions suggest that (a) under certain circumstances a disease may tend to die out and (b) under other circumstances a disease will reach an equilibrium after a series of epidemics of decreasing severity.[18]

A disease will, of course, die out if it does not reproduce itself, *i.e.*, if the bacterium is present only in the active case and each case does not, on the average, give rise to a second case. It is not unlikely that some diseases are dying out in this way; it has been suggested,[19] for example, that the decline in tuberculosis is due in part to inability of the disease to reproduce itself, a process accelerated by isolation of active cases. The second suggestion is of interest in connection with the epidemic periodicity of certain diseases such as measles, which appears in epidemic form usually at two year intervals. It is obvious, of course, that following an epidemic a new crop of susceptibles appears, and when their numbers reach a sufficiently high level, a new epidemic ensues and so on *ad infinitum*. It is unlikely, however, that these epidemic waves are damped as predicted by theoretical equations, for subtle variation in the complex of factors that are over-simplified as parameters will wipe out the damping effect. It will probably be many years before predictions regarding the future of infectious diseases attain a status better than that of guesses.

[17] For a discussion of this general problem see Lotka: *Elements of Physical Biology.* Williams & Wilkins Company. Baltimore. 1925.

[18] See also the discussion of harmonic analysis by Ziezold; Ztschr. f. Infektionskr., 1942, *124*:93.

[19] Frost: Amer. Rev. Tuberc., 1935, *32*:644.

Experimental Epidemiology.[20]—The information that may be derived from observation of naturally occurring disease, *i.e.*, descriptive epidemiology, is limited since the observer has no control over the process and for all practical purposes the experiment is carried out for instead of by him. In experimental epidemics, however, the conditions may be adjusted as desired and it may be anticipated that such experiments will be highly informative.

In recent years the study of epidemics of infectious disease developed in populations of laboratory animals under controlled conditions has been carried out by Topley, Greenwood, Wilson and others in England and by Webster and his colleagues in the United States.[21] These experimental epidemiological studies have been confined, for the most part, to the study of the dissemination of *Salmonella typhi-murium* (*aertrycke*), *Salmonella enteritidis*, *Pasteurella muriseptica* and the virus of ectromelia (a foot-pad disease of mice) among populations of mice. Mouse typhoid and pasteurellosis are regarded as analogous to human diseases such as typhoid fever in which there is an imperfect immunity and carrier state, and ectromelia as analogous to human diseases in which a solid immunity is developed, such as diphtheria. The extent to which the analogies may be carried, and to which the conclusions reached are applicable to the considerably more complex human population as it exists under natural conditions, is open to some question; nevertheless, these studies, as yet in their infancy, have yielded valuable information.

The experiments which have been carried out were of two general types. The closed epidemic was produced in a population of mice, often about fifty in number, by the introduction of infected animals. The other type of experiment was carried out in an infected mouse population recruited by continuous immigration, *i.e.*, mice were added at regular intervals, the rate varying from one mouse every three days to six mice per day. The results may be summarized briefly:

(1) The epidemic wave initiated in the closed population by the introduction of infected individuals closely resembled those observed in the human population.

(2) The effects of dispersal of the infected population of the closed epidemic into large or small groups at various times during the development of the epidemic were studied. The time at which the population was dispersed was found to be of primary importance; the later dispersal was effected, the less favorable the result, and after the peak of the epidemic wave was reached it continued unchecked even though the population was dispersed to individual mice. This is of particular interest since fleeing from an epidemic has been a popular method of escaping infection, *viz.*, the *Decameron*. In this connection the results of dispersal of children from the industrial centers of England in 1939–1940 may be noted. A sharp reduction of 40 per cent or more in diphtheria morbidity occurred in the evacuated towns together with an increase of 60 to 70 per cent among local

[20] See the general discussion by Topley: Proc. Roy. Soc., Ser. B, 1942, *130*:337.

[21] See the review by Greenwood, Hill, Topley and Wilson: Medical Research Council Special Report Series No. 209. 1936.

children in reception centers, the latter returning to normal within six months. Similarly, the biennial periodicity of measles was broken by this dispersal.[22]

(3) The effect of active immunization on the epidemic was not so great as might be supposed. If the mice were immunized prior to the epidemic, a favorable effect was apparent and more marked in ectromelia than in *S. typhi-murium* infections. Immunization undertaken after the initiation of the epidemic was without effect.

(4) In the mouse population recruited by continuous immigration a series of epidemic waves developed, the frequency of which was directly related to the rate of immigration. Thus, with a low rate, such as one mouse every three days, epidemic waves were separated by periods of remission in which no deaths occurred. As the immigration rate was increased, the waves occurred with greater frequency and with the addition of six mice per day there were no periods of complete remission, the epidemic waves taking the form of periods of increased mortality.

(5) With high immigration rates, the peaks and troughs in the daily death rate became less and less pronounced after a year or more, both the rate and total population tending to become invariant. Thus it was possible to approximate experimentally the damped periodicity and eventual equilibrium predicted on theoretical mathematical grounds (see above), and the experimental host population could be brought into approximate equilibrium with the parasite population.

(6) An inadvertent experiment with a population recruited by a high rate of immigration proved to be of considerable interest. The mouse population had reached the equilibrium indicated above by summer when there was a heat wave of unusual intensity in London. There were many nonspecific deaths (mice are highly susceptible to heat), followed by violently fluctuating specific mortality. In the course of time an equilibrium was again reached but at a considerably higher death rate and decreased numbers than formerly. The parasite was shown to be unaltered in virulence and the immigrants had not, of course, had the heat experience. Hence the host-parasite relationship *per se* was altered by an environmental "catastrophe." The relationship of these observations to the long term relation between the human population and infectious disease is of interest. If it be assumed that the day of the mouse is equivalent to thirty days in the life of man, over the four years taken by this experiment the following were observed: (a) a period of stability with low regular death rates and growing population (*ca.* 600 days) equivalent to a human experience of slightly less than fifty years—this, it may be noted, is longer than the twenty-five year period ending about 1830 during which the death rate from scarlet fever was relatively low; (b) a period equivalent to fifteen years of severe and repeated epidemic waves; and (c) a period equivalent to some thirty years of relatively high mortality in a more or less stable state of equilibrium in a population reduced in numbers—it may be noted that the post-1830 epoch of virulent scarlet fever lasted more than forty years.

(7) The mortality rates of the mice were intimately related to the length

[22] Stocks: Jour. Roy. Statistical Soc., 1941, *104*:311; *ibid.*, 1942, *105*:259.

of time that they were members of the infected population; the rate rose rapidly to a peak in the early days of cage age and, although it declined slowly with the passage of time, to a greater extent in ectromelia than in mouse typhoid or pasteurellosis, the survivors of one epidemic wave may be the victims of another. The removal at regular intervals of a number of mice equal to the number added at the same interval markedly altered the trend of these mortality rates; under these circumstances, the initial peak of mortality was lower, but the rapid decline was not apparent and the mortality rate remained at a high level.[23] Removal was of considerable advantage to the individual mouse; in general, the earlier the removal the greater the advantage, except during the decline of an epidemic wave, when isolation did not increase the chances for survival of individual animals.

Although the evolution of the single epidemic wave in the experimental studies closely resembles its counterpart in the human population, the inability to control experimental epidemics by immunization is at variance with observations on epidemic disease in human populations. Some human diseases, such as diphtheria and smallpox, can be restrained from assuming epidemic proportions by active immunization of a sufficiently large number of the individuals making up the population. Whether or not this and certain other minor discrepancies prove to be real or illusory with future work, a number of pertinent suggestions have come out of these investigations. The response of the experimental host population in terms of mortality rates and cage life expectation cannot be interpreted as yet in some cases; in others the interpretation is questionable. For example, the English workers feel that some of the data suggest alterations in virulence and "infectivity" of the bacteria, while Webster in the United States interprets these findings as indicative of a graded, inheritable resistance in the host population.

Some of the experiments, however, have yielded clear-cut results that bear directly upon the interaction of host and parasite populations in nature. Perhaps the most important of these is the experimental demonstration of recurring epidemic waves resulting from the continuous addition of new susceptibles to the infected population. In spite of the fact that such a sequence of events is predicted by theoretical epidemiology, as indicated above, the repeated flaring up of a disease of man or domestic animals, thought to be stamped out in the inter-epidemic periods, is most often taken as evidence of the reimportation of infection.[24] The experi-

[23] Greenwood, Hill, Topley and Wilson: Jour. Hyg., 1939, *39*:109.

[24] In this connection Major Greenwood (*Epidemics and Crowd-Diseases*. The Macmillan Company. New York. 1935) has made the following delightful suggestion: "... a majority of the officials who in different countries report upon epidemics among farm animals cannot bring themselves to conceive that upon a farm or within a district wherein a scheduled disease once existed, and was afterwards officially declared to have ceased to exist, it could start into life without a reimportation of *materies morbi*, these happenings must be explained by reimportation. We can always see a joke much more easily when it is against some poor foreigners rather than against ourselves. I advise an Englishman, therefore, to read the Dutch or German explanations of the recrudescence of foot-and-mouth disease and learn how the virus may be blown by the winds of heaven, or dropped on an island in the excreta of wild geese. A Dutchman might find explanations of the reimportation of foot-and-mouth disease to be found in English official documents equally bizarre."

mental demonstration of this phenomenon and the indicated futility of quarantine in the control of a disease which has become widely disseminated (only when a disease is not present, or present in limited effective foci, can quarantine and isolation procedures be effective) is, clearly, of considerable significance.

Epidemiological Data and Their Interpretation.—The purposes of epidemiological study are threefold: first, to indicate the nature of the infective agent, its source and modes of transmission when these are not fully established otherwise, *i.e.*, on an experimental basis; second, to extend this information into a corresponding general theory of the epidemiology of the disease; and third, to determine in detail the local conditions which favor or control the dissemination of the infection in a given area or community.

For these purposes four general types of information, usually simple in character but wide in extent, are required. First, the *area* in which the disease occurs and the regularity of its geographical distribution are highly informative. Thus, the general occurrence of a disease indicates that environmental conditions, including fauna, climate, etc., peculiar only to parts of the area are not essential to its transmission. Similarly, restriction of the disease within geographic limits indicates that special environmental conditions are necessary for its dissemination; these may include crowding, presence of an insect vector, water supplies, proximity to reservoirs of infection, etc. In general, a uniform distribution of the disease is indicative of a simple method of transmission, as in the case of measles, whereas an irregular distribution, such as that of spotted fever, implies a more complex process dependent upon a source of infection or conditions necessary to transmission that are correspondingly irregularly distributed.

Second, the *rate of prevalence* of the disease suggests the source of infection. A high rate, such as that of measles, indicates that the observed cases are the most important, if not the only source of infection. Conversely, sporadic distribution of widely separated cases implies the existence of a concealed reservoir of infection such as the casual or chronic carrier or some of the lower animals.

Third, the *seasonal distribution* is also informative when considered together with other epidemiological features of the disease. Thus, a seasonal occurrence may be consistent with hypothecated insect transmission or it may eliminate an insect vector as the sole transmitting agent. Similarly, the occurrence of water-borne typhoid fever in the late winter and early spring supports the hypothesis of preservation of typhoid bacilli in the cold and their liberation from contaminated watersheds in the first thaws.

Lastly, the *age distribution* of the disease is suggestive as a corollary to other epidemiological characteristics, and often aids in their interpretation. Thus, a disease occurring for the most part in the early years of life, such as measles or diphtheria, often shows differences in age incidence between urban and rural areas which are explained by the more frequent occurrence of immunizing infections, apparent or subclinical, in the more crowded areas. Since similar differentials are apparent in poliomyelitis, the age distribution of this disease, together with other of its epidemiological

features, supports the view that this disease is widely prevalent as a subclinical infection.

Epidemiological evidence, therefore, consists of a series of inter-related facts from which a conclusion or series of conclusions may be drawn. The first step in epidemiological procedure is necessarily the demonstration of associations between the frequency of occurrence of the disease and some condition or set of conditions, the second the ascertaining of the relationship of these associations with one another, and third their relations to the general epidemiological theory of the disease. To take a simple example, in milk-borne typhoid fever it may be ascertained that cases of the disease occur predominantly along the route of the milkman, that in families so supplied the cases occur among those who drink milk, that the cases appeared within a limited time to suggest simultaneous infection and that an employee of the dairy is a carrier of typhoid bacilli. Not only are these associations related to one another but they are consistent with the general epidemiological theory of typhoid fever, *viz.*, that transmission is basically a matter of the transfer of infected fecal material (or urine) from a case or carrier to the mouth of a susceptible person.

While this would appear self evident, there is an opinion of some prevalence that since epidemiological evidence is necessarily purely circumstantial, it cannot be conclusive. This is perhaps due in large part to failure to appreciate the development, often basically statistical and therefore mathematical, of the logical analysis, and the significance of the body of evidence as a whole. The method of analysis and interpretation of epidemiological evidence are identical with that of experimental evidence, the practical difference lying in the fact that, as pointed out earlier, the epidemic is an experiment done for rather than by the observer and cannot be manipulated.

The Control of Infectious Disease.—With an understanding, albeit admittedly imperfect, of the factors which determine the means and extent of the dissemination of infectious disease, comes the possibility of control. The variety of epidemiological types of disease and the individual variability within these broad groups make for differences in control measures, and the control of a given disease under given circumstances almost always constitutes a special case. Certain generalizations may, however, be made which will serve to indicate the complex nature of the problems of the control of the spread of infection and provide a point of departure for consideration, in later chapters, of specific diseases.

In general, control measures may be regarded as corollaries of the factors involved in the transmission of infection and, therefore, fall into two groups: one including those which raise the resistance of the individual and, through him, of the population; and the other, those designed to alter the relationships of the individual members of the population to one another in such a way that the opportunities for the dissemination of the infectious agent are reduced. Very often both types of control measures are used, their relative efficiency and practicality varying from one disease to another.

Diseases of lower animals which are transmitted either directly or indirectly to man involve a third population, the animal reservoir of infection.

When transmission is from animal to man and occurs only rarely from man to man, three methods of control are possible. First, the disease may be controlled in the animal; second, the relationship between the human and animal populations may be altered in such a way that transmission of infection cannot take place; and third, the resistance of the human population may be raised by immunization procedures. The relative efficacy of these methods of control is variable and depends upon the disease. Rabies, for example, can be controlled in the dog population, and, in fact, has been entirely eliminated in England by rigid regulation, but neither mass immunization of the human population nor alteration of the relationship between dog and human populations is practical. In the case of bovine tuberculosis and undulant fever, however, disease may not only be controlled in the animal population, but the relation between human and animal populations is readily altered by interposing the barrier of pasteurization. In other instances in which the reservoir of infection is a wild animal, immunization, when a solid immunity can be obtained, of the limited number of individuals who have contact with such infected populations is satisfactory.

The insect-borne diseases present still other problems in control in that the link between man and animal or man and man is a living organism. In general, control of the insect population or of the disease in the insect will result in control of the disease in man. The infections transmitted by insects are, for the most part, rickettsial, protozoan and virus diseases; few bacterial diseases are insect-borne, presumably because in many of the blood-sucking insects the intestinal tract is actively bactericidal and most species of bacteria are rapidly destroyed there. The mechanical transmission of bacterial infection by insects is, then, relatively rare and, except under unusual circumstances, of no great importance. Two species of pathogenic bacteria are, however, resistant to this bactericidal activity: the bacilli of plague and tularemia multiply in the rat flea (*Xenopsylla cheopis*) and the deer fly (*Chrysops discalis*) respectively. When the microorganism either multiplies or completes a portion of its life cycle in the insect vector, the insect is infective for the duration of its life; such is the case in yellow fever, typhus fever, malaria and other diseases. The problems of control of these diseases are the problems of the control of the insect population, either indirectly through the insect's animal host as in bubonic plague or directly as in malaria. A further complication is introduced in the case of spotted fever. The tick (*Dermocentor andersoni*) undergoes an incomplete metamorphosis, and the infection is "hereditary," *i.e.*, transmitted to the second generation. There is, in consequence, not only an animal reservoir of infection but also a second reservoir in the form of infected ticks.

Diseases Transmitted Directly from Man to Man.—The diseases transmitted directly from man to man by contact or by air-borne infection are by far the most difficult to control. The mechanism of transmission is, of course, very simple and does not offer the possibilities for interference that may be taken advantage of in the more complex processes. Furthermore, the practices and habits of the human population that make possible transmission by these means are a part of day-to-day existence in the life of

man. Thus the aggregation of human beings under crowded living conditions in urban areas, and the theatres, schools, churches, and other public meeting places cannot be dispersed or eliminated for the control of air-borne infection. Similarly, prevention of the spread of venereal disease is theoretically possible but relatively unsuccessful in practice.

In the past, therefore, control of disease spread by these means has been dependent upon effective active immunization procedures. Diphtheria and scarlet fever may be so controlled by the immunization of a reasonably large part of the population, but influenza, the common cold and others continue to be widespread.

With the elucidation of the mechanisms of air-borne infection and the development of aerosols (p. 70) and ultraviolet irradiation for the destruction of air-borne bacteria, it seems possible that the spread of air-borne infection may be controlled. Thus, the establishing of air sterilization in public gathering places of various kinds, possibly in the air conditioning systems, as well as in hospital wards may be effective.

The control of the spread of a given disease is, in practice, complicated by two important factors. First, a disease is often transmitted in more than one way. This variation may be within narrow limits; for example, an insect-borne disease may be transmitted by several species of insects which differ from one another in distribution, breeding habits and the like. In extreme cases a disease may fall into more than one epidemiological type; bubonic plague, for instance, which is transmitted from the rat to man by the rat flea, may assume a pneumonic form transmitted by infective droplets and become independent of the rat population. Secondly, infection may be carried and transmitted, not only by clinically recognizable cases of disease but also by cases before clinical symptoms appear; by individuals who have a disease in such a mild form that it is not recognized; and by healthy individuals who carry the infection either transiently or semipermanently. For example, while it is not difficult to prevent epidemics of water-borne typhoid fever, the disease remains endemic in a community as a result of the dissemination of the bacilli from ambulatory cases and carriers, and this so-called "residual typhoid" is extremely difficult, if not impossible, to eradicate.

It will be clear from the foregoing discussion that the transmission of infection is indeed a highly complex matter. Each disease is a special case of the host-parasite relationship which is not infrequently complicated by the interaction of two or more host populations. The elucidation of the interrelationships of these groups is a necessary preliminary to the understanding of the mechanisms operative in the dissemination of disease.

CHAPTER 10

THE BACTERIOLOGY OF WATER AND SEWAGE

Of the interrelationships between the members of human populations that facilitate the transmission of infection, those arising as a consequence of common water supplies and the group disposal of sewage are among the most important. Transmission of disease by this means is, of course, confined to diseases of the so-called enteric group, in which infection takes place via the gastro-intestinal tract and the causative microorganisms are discharged with the feces. Infection is, therefore, the result of a direct connection between infectious fecal material and the mouth of a susceptible person, and when that connecting link is a common water supply, as it frequently is, outbreaks of cholera, typhoid fever and the like occur. This link is, however, readily broken; the great water-borne epidemics are rapidly becoming a thing of the past as a consequence of the utilization of effective control measures, and present day water-borne epidemics are indicative, not of a lack, but of a failure to make use of existing knowledge.[1]

WATER[2]

Because of water-borne disease and the obvious desirability of its control, studies of the bacteriology of water have been directed, for the most part, toward its sanitary aspects. The best single criterion by which the sanitary quality of a water may be judged is, clearly, the kind and numbers of bacteria that are present in it. If it were possible, as a routine procedure, invariably to detect the presence of the appropriate disease-producing bacteria, it would be unnecessary, from the sanitary point of view, to take the non-pathogenic forms into consideration. This is, however, not the case; a judgment of the sanitary quality of a water cannot be made on the basis of failure to find a microorganism such as the typhoid bacillus in that water. This bacterium, for example, is usually so outnumbered by similar forms such as the colon bacillus that its isolation and identification require enrichment cultures in selective media and other time-consuming procedures and even then are not always successful, and a negative finding is of doubtful value.

It is legitimate to assume, however, that when a water is polluted with human fecal material, it is probable that it contains bacteria which cause enteric infection. This probability becomes a certainty when the fecal material is pooled, as in the sewage of a community, because of the ubiquitous presence of healthy carriers of typhoid bacilli and similar microorganisms. Since it is impractical to isolate these pathogenic bacteria them-

[1] Gorman and Wolman: Jour. Amer. Water Works Assn., 1939, *31*:225.

[2] Prescott and Winslow: *Elements of Water Bacteriology.* 5th ed. John Wiley & Sons New York. 1930.

selves as a routine measure, some indicator of fecal pollution will serve equally well as a criterion of the sanitary quality of a water. The question, which was stated many years ago,[3] becomes this: Are there types of bacteria which are never present in natural waters free of pathogenic bacteria but which may always be found in water polluted with human fecal material and therefore probably with pathogenic bacteria? If so such types of bacteria can be used as indicators of pollution. The answer to this question involves a consideration of the bacterial flora of natural waters, including both bacteria which are native inhabitants of water and those microorganisms whose presence is a consequence of contamination from external sources.

Bacteria Native to Natural Waters.—The bacteria whose native habitat is water are not well known,[4] in part because many of them are difficult to grow on laboratory media. There is no question, however, of the existence of a bacterial flora normal to and characteristic of natural waters. The types of bacteria which make up this natural population may be considered briefly:

(1) *higher bacteria*, frequently the sheathed forms assigned to the *Chlamydobacteriales* and including sulfur, iron and other forms;
(2) the *Caulobacteria*, a relatively recently described group of "stem" bacteria which occur in lakes and other bodies of water attached to some inanimate object;
(3) the *spiral forms*, which are frequently found in great numbers in water, some of which may be very large, 20 to 30 μ in length, as compared with the parasitic spirilla;
(4) a variety of *bacilli*, including
  (a) pigmented forms such as *Bacterium prodigiosum*, *Bacterium violaceum*, *Bacterium aurescens*, and others,
  (b) various non-pigmented forms such as
    1. the fluorescent bacteria—*Pseudomonas fluorescens*,
    2. certain of the sulfur bacteria,
    3. thermophiles,
    4. aerobic, spore-forming bacilli of uncertain taxonomic position;
(5) *coccus forms*, both
  (a) pigmented, generally yellow—very often *Sarcina lutea*, and
  (b) non-pigmented—*Micrococcus aquatilis*, *Micrococcus candicans*, and others;
(6) *nitrogen-fixing bacteria*—*Azotobacter aquatile* in particular;
(7) *nitrifying bacteria*—both *Nitrosomonas* and *Nitrobacter*.

These water bacteria are found in fresh water in swamps, streams and lakes. The bacterial populations of salt waters have not been studied until recent years, but it appears[5] that the sea contains similar bacteria, in-

[3] *Cf.* Jordan in Thirtieth and Thirty-first Repts. Mass. State Bd. of Health, 1898, 1899.

[4] *Cf.* the review by Baier: *Studien zur Hydrobakteriologie stehenden Binnengewässer.* Arch. f. Hydrobiol., 1935, *29*:183, also the studies of Taylor: Jour. Hyg., 1940, *40*:616; *ibid.*, 1941, *41*:17; *ibid.*, 1942, *42*:284.

[5] ZoBell and Feltham: Bull. Scripps Inst. Oceanog., Tech. Series, 1934, *3*:275; Waksman, Hotchkiss, Carey and Hardman: Jour. Bact., 1938, *35*:477.

cluding the nitrogen-fixing forms, the fluorescent bacteria, various pigmented forms and the like. The water bacteria bear the same relation to the constant and recurring transformations of organic matter that take place in natural waters as they do to the same processes in the soil and are, therefore, an integral part of aquatic life.[6]

Some, but by no means all, of the water bacteria can be cultivated on laboratory media. In any case no one medium suffices; the nitrifying bacteria, for example, cannot be grown in the presence of organic matter. Since the bacteria found on plates are of necessity only those that will grow upon the medium used, it will be clear that the value of plate counts as estimates of the numbers of these microorganisms is questionable. Enrichment cultures are sometimes useful, and it may be noted parenthetically that the simple holding of a water sample for a period of hours will frequently result in a marked increase in numbers. Considerable information has been obtained in recent years by a method of "slide culture" in which a clean glass slide is suspended in the water for several days, removed, stained and studied microscopically.

Bacterial Contamination of Natural Waters.—In addition to the native water bacteria, a given water may, and usually does, contain a variety of bacteria as contamination from external sources. These sources are two—the air and soil, and human excreta.

Bacteria from the Air and Soil.—The number of bacteria in the air bears, as might be supposed, a close relation to the quantity of larger suspended particles or "dust." There are fewer bacteria in the air of the country than of the city, fewer in mountain air than in the air of the lowlands, and the air in midocean is nearly bacteria-free. Under appropriate circumstances bacteria suspended in the air are those expelled from the upper respiratory tract of human beings and are of no small significance in the transmission of "droplet infection," but in so far as air in general is concerned this is an insignificant and relatively rare occurrence. Pathogenic microorganisms, such as the tubercle bacillus and the pyogenic cocci, have been found in the air of hospitals and sickrooms, but as a rule pathogenic bacteria in dry dust are exceedingly rare. The kinds of microorganisms in the air vary somewhat in different localities, but certain forms are generally present. Molds and yeasts are quite common and in some instances outnumber the bacteria. These include various species of molds such as *Penicillium glaucum* (the blue-green mold) and various yeasts, many of them the pigmented (red) torulae. *Bacillus subtilis* and related forms, together with the various micrococci, often pigmented, are almost universally present. These organisms may be isolated from the air by various filtering devices and most efficiently by the air centrifuge of Wells. They find their way into natural waters through settling out or are swept down by rain.

Many of the bacteria present in the air, particularly the aerobic sporeformers, are essentially soil forms blown up in dust and able to survive drying. The soil itself contains tremendous numbers of bacteria; a gram of average field soil probably contains 100,000,000 to 50,000,000,000 living

[6] *Cf.* Henrici in *Problems of Lake Biology.* Amer. Assn. Advancement of Science, Publication No. 10. 1939.

bacteria, most of which are found in the upper six inches of soil, few being found in undisturbed soil below a depth of four to five feet. The great majority of these microorganisms are native to the soil and include the nitrogen-fixing and nitrifying bacteria, bacteria of the amylobacter group and a variety of forms whose biochemical activities are an integral part of the mechanism of the decomposition of organic matter in the soil.[7] A small part of the native bacterial flora of the soil consists of potential pathogens such as *Clostridium tetani*, *Clostridium edematis* (the bacillus of malignant edema), *Clostridium botulinum* and others.

Bacteria other than those which make up the normal flora of the soil may be present as contamination. For practical purposes the pathogens that may be present may be regarded as coming from one of two sources, the flesh of animals and of persons who have died of infectious disease, and the excreta of human beings. In the first instance only one organism, the anthrax bacillus, is of significance, for other bacteria causing diseases of animals and man, such as the causative agents of tularemia, plague, spotted fever and other diseases of animals, together with the bacteria of human disease such as the diphtheria bacillus, streptococci, etc., do not survive long in the soil (p. 132).

In the case of the anthrax bacillus, however, the spores are able to survive for long periods of time, perhaps years, and are brought to the surface from buried bodies by earthworms. Pastures may, then, remain infective for cattle for long periods of time.

The pathogenic bacteria contained in human excreta are, of course, those microorganisms which leave the body via the intestinal tract, *i.e.*, those causing the enteric diseases. These bacteria do not multiply in the soil but their vitality may be considerably prolonged, possibly for two to three months.

It will be clear that bacteria present in the air and in the soil have relatively ready access to bodies of water and contamination may take place either more or less continuously or at irregular intervals under certain unusual conditions, as during and immediately after heavy rains. The contribution of air and soil, the latter in particular, to the bacterial flora of water is, then, considerable. Since the bacteria native to water are not pathogenic, what of this contamination with respect to the sanitary quality of a water? Some of the potential pathogens, *e.g.*, the bacilli of tetanus and malignant edema, are not infective when taken by mouth, and others, even though they survive for sufficient periods, rarely produce infection by this route. The normal portal of entry of the enteric bacteria is, however, the gastro-intestinal tract, and it is these microorganisms which constitute the significant contribution of contamination through the agency of soil.

The intestinal bacteria may be washed directly into lakes and rivers or other bodies of water by heavy rains; hence the presence of excreta on water sheds and consequent contamination of impounded waters is of considerable practical importance. In many instances, particularly in the case of shallow wells and the like, typhoid and other bacilli enter the water

[7] Waksman: *Principles of Soil Microbiology*. Williams & Wilkins Company. Baltimore. 1st ed., 1927; 2nd ed., 1932.

supply from privies, latrines and similar devices via ground water. The distance over which such contamination can travel is a function of the rate of death of the bacteria and the rate of ground water flow. The latter is obviously dependent upon many factors such as the amount of rainfall, the local geological formations and the like, and each instance of contamination or possible contamination must be considered individually. Fecal bacteria have been found to penetrate from 100 to 200 feet in ground water.[8] In general fine soils and sand tend to impede their progress to a greater extent than coarse sand and gravel. The rock formations may be of considerable significance; sandstone, for example, filters out bacteria, while limestone tends to erode with the formation of direct communicating channels, and wells drilled into such formations may contain typhoid bacilli which entered the water many miles away and are, therefore, always to be regarded as dangerous.

Contamination by Human Excreta.—Contamination of water by human excreta may take place, not only indirectly through the agency of soil as noted above, but also directly. Such direct contamination is, for the most part, a consequence of human population densities and urban organization, and takes the form of dumping of sewage of one community into a body of water which serves as a water supply to another. Whether directly or indirectly contaminated, such waters contain not only the native bacterial flora supplemented by microorganisms from the soil, but also the bacterial flora of the human intestine. The contribution of the last consists primarily of *Bacterium coli* in very large numbers, together with *Clostridium welchii*, *Streptococcus fecalis* and the various intestinal pathogens.

Factors Influencing the Kinds and Numbers of Bacteria.—The numbers of bacteria that may be found in a given water are dependent primarily upon the type of water, whether it is a *surface water*, such as that found in streams, lakes and shallow wells, or a *deep water* from deep driven wells. In the first instance opportunities for contamination are great and, as might be expected, many bacteria are present. The water from deep wells, on the other hand, has undergone an effective filtration in order to reach the deeper strata in which it is obviously not subject to any extensive contamination; hence relatively few bacteria are found.

A variety of environmental factors influences the bacterial content of water; chief among these are the amount of organic matter present and the temperature. In general, the more nutriment there is present in the form of organic matter, the greater the number of bacteria. Low temperatures are not conducive to rapid growth and tend to keep the numbers of bacteria down, a factor that favors the survival of pathogens such as the typhoid bacillus which are unable to multiply in any case. Higher temperatures result in an increase in bacterial numbers in the presence of sufficient organic matter, but if the supply of nutriment is not great, after a preliminary increase during which the food supply is exhausted, the numbers fall below the initial level. Other environmental factors are not infrequently influential in determining the types of bacteria present in a water; thermophiles will, of course, predominate in hot springs and sulfur bacteria in

[8] See, for example, the studies of Caldwell: Jour. Inf. Dis., 1938, *62*:225, 272.

sulfur springs; and the acidity of many natural waters results in a limited flora of acid-resistant bacteria.

Bacteria in Ice.[9]—Although it is difficult if not impossible to sterilize a substance by exposure to low temperatures, many of the bacteria present are killed, only the resistant cells surviving. The great majority of bacteria in water are killed by freezing. Hence ice always contains but a fraction of the number in the water from which it was formed. Over 90 per cent both of the ordinary water bacteria and of typhoid bacilli die within a few hours, and a progressive decline in numbers then takes place, less than 1 per cent of typhoid bacilli surviving at the end of a week of freezing. Ice stored for six months is practically sterile. Outbreaks of typhoid fever have rarely been traced to the use of ice, although in a few instances the evidence of ice transmission seems quite conclusive. Danger of typhoid infection from the use of ice in drinking water is, in the absence of direct contamination of the ice during handling, always less than from the use of water from the same source as the ice.

The Bacteriological Analysis of Water.[10]—It will be apparent from the above discussion that the bacteria whose presence in water is a consequence of fecal pollution are not present in uncontaminated water and are sufficiently different from the native water bacteria that they may be readily distinguished. Of these bacteria *Bacterium coli* is present in greatest numbers, while *Streptococcus fecalis* and *Clostridium welchii*, although constantly present, are usually not so numerous. It would appear, therefore, that any of these microorganisms could be used as an indicator of pollution. Of these *Bact. coli* is the most satisfactory, although both *Str. fecalis* and *Cl. welchii* have been used in Europe. The streptococci are, however, sometimes difficult to differentiate. *Cl. welchii* has the disadvantage that its spores remain viable over long periods of time in contrast to *Bact. coli* which, although more hardy than the typhoid bacillus, dies out in time; hence *Cl. welchii* does not allow the differentiation of recent and old pollution. The quantitative relations between the coliform bacteria and the enteric pathogens are discussed at some length by Kehr and Butterfield.[11]

The bacteriological examination of water for the presence of *Bact. coli* rests upon the fact that this microorganism ferments lactose. The standard procedure for the examination has been prepared jointly by the American Public Health Association and the American Water Works Association, and is revised at frequent intervals.[12] Briefly, it consists of three parts, (1) the presumptive test, (2) the confirmed test and (3) the completed test. In the first, lactose broth is inoculated with decimal dilutions of the water sample, commonly 10 cc., 1 cc. and 0.1 cc. (expressed as dilutions these are, respectively, 0.1, 1 and 10). The volume of the smallest inoculum producing

[9] See Jensen: Food Research, 1943, *8*:265.

[10] For British practice, which differs slightly from American, see Ministry of Health Rept. No. 71, *The Bacteriological Examination of Water Supplies.* His Majesty's Stationery Office. London. 1939.

[11] Kehr and Butterfield: Pub. Health Repts., 1943, *58*:589.

[12] This procedure may be found in detail in *Standard Methods of Water Analysis*, American Public Health Association, 8th ed., 1936. and in condensed form in most of the standard laboratory manuals.

fermentation provides a crude approximation of the numbers of *Bact. coli* present in the water. A more accurate estimate may be obtained by inoculating five tubes with each dilution and calculating the most probable number of *Bact. coli* on the basis of the number of tubes in which fermentation occurs. The *confirmed test* consists of the inoculation of a specified selective medium such as Endo or eosin-methylene blue (EMB) plates, brilliant green lactose bile broth, crystal violet lactose broth, fuchsin lactose broth or formate ricinoleate broth. The appearance of typical *coli* colonies on the plates or fermentation in the selective lactose broth constitutes a positive confirmed test. In the *completed test* one or more typical colonies are picked from an Endo or EMB plate inoculated either from the original lactose broth culture or from the secondary selective medium showing fermentation and are transferred to an agar slant and a lactose fermentation tube. After incubation the slant culture is smeared and stained and examined for the gram-negative non-spore-forming rods of *Bact. coli.* If the culture is found to be morphologically *Bact. coli* and the lactose is fermented, the completed test is positive.

The Coliform Bacteria.—Although *Bact. coli* is readily distinguished from the native water bacteria, the closely related *Bacterium aerogenes* is found in grains and elsewhere in nature though not, it may be noted, in virgin soils. The two bacterial species may be differentiated by a number of tests (p. 275), but these are not a part of the standard method of water examination; hence the gram-negative, lactose-fermenting bacteria whose presence is determined are more properly termed *coliform bacteria* rather than *Bact. coli.*

The Sanitary Significance of Coliform Bacteria.—In recent years there has been a tendency in sanitary water analysis to emphasize the distinction between *Bact. coli* and *Bact. aerogenes*, the presence of the latter being considered by many to have little or no sanitary significance. There is no doubt that *Bact. coli* is more predominantly of "fecal origin" while *Bact. aerogenes* is found in greater relative abundance in soil than in sewage. For this reason it is maintained that a predominance of *Bact. aerogenes* over *Bact. coli* in a water supply is more indicative of soil contamination (the *coli* presumably of animal origin) or past pollution than of recent pollution. Particularly in tropical countries—where, however, intestinal disease is rife—it is urged that the presence of microorganisms of the *Bact. aerogenes* type in drinking water is of little sanitary importance. The ecology and significance of the different types of coliform bacteria found in water are reviewed and considered in detail by Taylor.[13]

It should be remembered, however, that coliform bacteria of all kinds are practically absent from virgin soils and from pure spring and surface waters, and that while *Bact. aerogenes* and intermediate forms are not present in feces in as great numbers as *Bact. coli*, their presence may nevertheless be demonstrated by appropriate methods.[14] Even if it is true that *Bact. aerogenes* is somewhat more resistant then *Bact. coli*, and hence may survive in soil or water long after the latter has disappeared, the fact that

[13] Taylor: Jour. Hyg., 1942, *42*:23.

[14] Gray: Jour. Hyg., 1932, *32*:132; Bardsley: Jour. Hyg., 1934, *34*:38

its presence is not reliable evidence of *recent* pollution may not be as decisive as sometimes assumed. Experience in water examination has shown that it is not safe to disregard the warning of potential danger conveyed by evidence of soil washings and "past pollutions." It is unwise, therefore, in routine water analysis, to place too much stress upon the differentiation between *Bact. aerogenes* and *Bact. coli*, at all events until the practical value of such differentiation can be clearly demonstrated.

Plate Counts.—It is usually desirable to have an approximate measure of the total number of bacteria in drinking water, not because the sanitary quality of a water can be judged on this basis alone, but because such information frequently has ancillary value. The counts obtained by quantitative dilution and plating are, it must be remembered, those of the microorganisms that will grow on the medium used, other bacteria being quite inapparent by this method.

Two series of plates are poured, one in which the medium is nutrient gelatin and the other nutrient agar, or both may be nutrient agar. The gelatin plates, or one set of agar plates, are incubated at 20° C. and the agar plates at 37° C.[12] In general the native water and soil bacteria grow best at 20° C.; in some cases they do not grow at all at 37° C.; and bacteria of animal origin grow most rapidly at body temperature. The relative numbers of microorganisms growing at the two temperatures are, then, at times suggestive of the origin of the bacteria found.

Chemical Analysis.—The analysis for appropriate chemical compounds frequently is of value as an adjunct to bacteriological analysis in the determination of the sanitary quality of a water.[12] Pollution by sewage, for example, adds complex compounds, protein, carbohydrate and fat, to the water, and the amount and state of the decomposition products of these substances may serve as an index of the degree and time of pollution. Ammonia, nitrites, nitrates, chlorides and albuminoid nitrogen are usually determined. It may be noted that the chemical analysis of water is frequently made in connection with hardness, turbidity, taste, smell and similar features, which, while often of considerable industrial or esthetic significance, are of no sanitary importance.

The Assay of the Sanitary Quality of Water.—The means by which the sanitary quality of a water is judged may be summarized briefly:

(1) The bacteriological analysis, including both
 (a) the presence or absence of coliform bacteria and
 (b) the number and type of bacteria present;
(2) the type of water, whether surface or deep;
(3) the local conditions; and
(4) chemical analysis.

Of these the presence and numbers of coliform bacteria are the most important, and it must be remembered that the relative abundance, rather than the presence, of these microorganisms is the essential feature of the test. The discovery of a single colon bacillus in 50 cc. of water, or even occasionally in 5 cc., affords no reasonable ground for suspicion of the water. The possibility of sporadic contamination with colon bacilli derived not from man but from domestic animals or birds must be kept in mind.

Manured fields and pastures, filled with grazing cattle or sheep, are likely sources of colon bacilli and may give rise to mistaken inferences if the environmental examination of a water supply is neglected. Knowledge of such local conditions as well as the type of water may, then, be essential to the interpretation of the bacteriological findings. Chemical analysis may be of considerable help in some instances, but in general it is not essential.

Bacteriological Standards.—The question of the significance of the bacteriological findings brings up the matter of standards to which a water suitable for drinking should conform. Now it will be clear that, although considerable numbers of colon bacilli in a water are always suggestive of fecal contamination by man or animals, a standard is necessarily a minimum which is inherently difficult to define under the circumstances. In the United States the only such standard is that of the Treasury Department for drinking water on common carriers:[15]

(1) Of all the standard (10 cc.) portions examined . . . not more than 10 per cent shall show the presence of organisms of the *Bact. coli* group.

(2) Occasionally three or more of the five equal (10 cc.) portions constituting a standard sample may show the presence of *Bact. coli*. This shall not be allowable if it occurs in more than:

(a) five per cent of the standard sample when twenty or more samples have been examined;

(b) one standard sample when less than twenty samples have been examined.

Note.—It is understood that in the examination of any water supply the series must conform to both the above requirements.

An approximate and qualified standard has been prepared by the English workers[16] which, in a summarized form,[17] has considerable illustrative value but is not to be taken as absolute:

	deep waters	surface waters
Plate count, agar, 3 days, 22° C.	10–200 per cc.	50–500 per cc.
Plate count, agar, 2 days, 37° C.	1–10 per cc.	5–30 per cc.
Bact. coli	<1 per 100 cc.	<5 per 100 cc.
Fecal streptococci	<1 per 100 cc.	<5 per 100 cc.
Cl. welchii	<1 per 1000 cc.	<5 per 1000 cc.

The two kinds of water given above represent, as deep water, a water that is satisfactory for human consumption and, as surface water, a water that, while probably not dangerous, is open to some suspicion. In the absence of further information, however, a water cannot be judged on the basis of the bacteriological findings only; this is evident in the case of the above "surface waters." Current American views on the question of standards are discussed in the Journal of the American Water Works Association.[18]

The Purification of Water Supplies.—When, by bacteriological examination or otherwise, a water is known to be unsafe for consumption, the

[15] Public Health Reports, 1925, *40*:693; *ibid.*, 1943, *58*:69.

[16] *The Bacteriological Examination of Water Supplies*. Ministry of Health Series No. 71. His Majesty's Stationery Office, London. 1934.

[17] Taken from Topley and Wilson: *Principles of Bacteriology and Immunity*. 2nd ed., 1936. William Wood & Company. Baltimore. p. 1583. These writers tentatively offer, for illustrative purposes only, specific standards based on British practice.

[18] Jour. Amer. Water Works Assn., 1943, *35*:93, 135.

question arises as to ways and means of artificial purification. There are a number of useful methods of purifying water, differing according to the amount and character of the water to be treated, which may be summarized as follows:

(1) mechanical methods
 (a) storage
 (b) filtration
  1. slow sand filtration
  2. coagulation and rapid sand filtration
(2) chemical methods
 (a) large scale—hypocholorites and liquid chlorine
 (b) small scale—hypochlorite, ultraviolet light, ozone, etc.

Of these, storage is not generally regarded as a method of water purification, though the numbers of bacteria are usually greatly reduced in impounded waters because of the exhaustion of the food supply and the consequent death of bacteria and settling, not so much of bacteria alone as of suspended matter which carries down bacteria with it. The partial

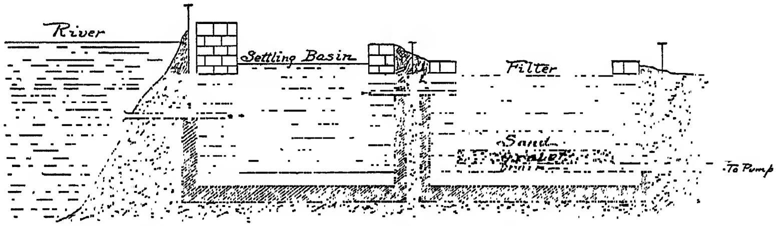


Fig. 28.—Cross-section of filter plant (after Hazen).

removal of suspended matter is frequently desirable, particularly with turbid waters, and may be carried out by allowing the water to remain in a settling basin for a time.

Slow sand filtration is one of the earliest and most effective methods of water purification and is in use in many European and some of the older American cities. These sand filters are constructed so that the water passes through 1 to 5 feet of sand supported upon graded layers of gravel (see Fig. 28). The rate of filtration must be accurately regulated and the efficiency of operation controlled by frequent bacterial tests of the effluent. Such filters are highly effective. Bacteria are removed, not to any great extent by mechanical straining out, but through a biological mechanism in which the activity of protozoa is an important feature. The passage of water through these filters is necessarily a relatively slow process and, in consequence, relatively large areas are required, which, for financial or other reasons, are no longer available in large American cities. Few of these filters have been constructed in recent years, and the use of rapid sand filters is becoming common.

The rapid sand filters, which may be used with turbid waters that would clog a slow sand filter, are frequently employed in conjunction with "coagulation," the addition of such substances as aluminum or ferric sulfate.

which form flocculent precipitates (the hydroxides). The precipitate carries down most of the suspended matter and, of course, many bacteria, and is readily filtered out, yielding a clear effluent containing relatively few bacteria. Laboratory experiments on centrifugation of dilute suspensions of *Bact. coli* in the presence of kaolin or infusorial earth have indicated that 70 per cent of the viable cells are found in the sediment.[19] Such filters must be cleaned from time to time, a process that is seldom required with slow sand filters. They make possible, however, the treatment of a large quantity of water on a relatively small filtering area.

Destruction of pathogenic bacteria in water supplies is most often brought about by treatment with germicidal chemicals. Of these, hypochlorite (calcium hypochlorite or bleaching powder) was at one time used widely in the treatment of municipal water supplies, and it still has its uses, although it has been largely superseded in large scale treatment by liquid chlorine, which is supplied in cylinders under pressure. Chlorine is added directly to the water through an automatic feeding device in accurately measured amounts which are determined by the character of the water. In general, the greater the amount of extraneous organic matter present, the greater must be the amount of chlorine added. The amount of chlorine taken up is termed the chlorine demand, and the point at which the residual chlorine or available chlorine becomes proportional to the added chlorine is called the "break point" in the chlorine demand curve. When liquid chlorine is added to ordinary surface water, clear and not highly contaminated, in the proportion of about 0.5 to 1 part of "available chlorine" per million gallons, the ordinary intestinal bacteria are destroyed, including such pathogenic forms as the typhoid bacillus.[20] The tastes and odors in chlorinated waters may be due to an overdose of chlorine caused by inadequate control methods, or to the action of chlorine upon compounds present in the water, commonly as industrial wastes. Excess chlorine and tastes caused by chlorination can often be removed by dechlorination with SO_2 or treatment with $KMnO_4$ or activated carbon. The bactericidal activity of chlorine may be prolonged, particularly in waters containing considerable organic matter, by the simultaneous addition of liquid ammonia with the formation of chloramines.[21] The introduction of chlorination of municipal water supplies has, in practically every instance, resulted in marked reductions in the incidence of enteric infection, typhoid fever in particular, in this country. The addition of chlorine or other bactericidal agents is not a "cure-all" however. When a water is sufficiently heavily contaminated bacteria embedded in particles of organic matter are not killed even in the presence of available chlorine. It is generally agreed that a content of more than 50 coliform bacteria per cubic centimeter indicates pollution too great for successful chlorination.

It may be noted that frequently a combination of filtration and chlori-

[19] Schulhoff and Huekelekian: Jour. Amer. Water Works Assn., 1936, *28:*1963.

[20] See, for example, the studies of Levine, Heller and Bender: Jour. Amer. Water Works Assn., 1942, *34:*1787.

[21] The chlorine-ammonia treatment is discussed in Jour. Amer. Water Works Assn., 1941, *33:*2079.

nation is desirable particularly with rapid sand filters. Not only does a preliminary filtration have esthetically desirable features in the case of turbid waters, but considerably less chlorine is required for treatment than would have been the case had not the greater part of the suspended material been removed.

The chemical treatment of water on a small scale does not always involve expense as a primary consideration and hence other methods are not infrequently used. The treatment of swimming pool water by ultraviolet irradiation adds no taste or odor to the water and, although considerably more expensive than chlorination, can be used because the scale of operation is small. Ozone, which is strongly bactericidal, is also relatively expensive and is not commonly used in this country but is used to an appreciable extent in Europe.[22] Chlorination is, of course, always available. It is carried out by the addition of hypochlorite when the installation of liquid chlorine apparatus is not desirable, as, for example, in the treatment of a contaminated cistern.

At times the treatment of water becomes an individual matter, as in the case of an army in the field or when a public supply is known to be impure. In the first instance the water may be treated by the addition of hypochlorite in the form of bleaching powder or a solution of sodium hypochlorite, or by the addition of a small amount of iodine, one part of Lugol's iodine solution to 1000 parts of water. In the home, filters, such as the Berkefeld or Chamberland bougies, may be used, but filtration is slow and care in operation and frequent cleaning are necessary. The simplest and best method of water treatment for the family or individual, however, is simple boiling. Boiling for five minutes is quite sufficient to destroy with certainty the typhoid bacillus and allied forms as well as the cholera vibrio. When water-borne disease is prevalent, or when a water supply is notoriously impure or exposed to chance of infection, boiling is the only wholly safe procedure.

SEWAGE

Sewage is best regarded as the used water supply of a community and as such is a dilute solution of fecal matter and other wastes. From the hygienic point of view it is an important vehicle in the transmission of enteric infection; hence the manner of its disposal is of considerable significance. The mechanisms of sewage disposal have as their objects first, the ridding of a community of an ever-present volume of waste and, second, disposal in such a manner that it is not dangerous to other communities.

The complex organic compounds present in sewage undergo the same processes of decomposition that are involved in the breakdown of dead organic matter in nature and are a part of the so-called cycles of elements such as nitrogen, phosphorus and the like. Any type of sewage treatment, then, is nothing more than a mechanism for bringing about or accelerating these transformations. The organic compounds are first broken down to amino acids, monosaccharides and the like, which are eventually oxidized completely to carbon dioxide and water in the case of carbon and hydrogen,

[22] See the review on ozone treatment by Hann: Jour. Amer. Water Works Assn., 1943, *35*:585.

and to nitrite and nitrate in the case of nitrogen. Bacteria are the active agents in this decomposition and oxidation, the mechanisms of which have been discussed earlier (Chapter 5). Although essentially simple in principle, sewage treatment is in practice a complex problem which cannot be considered at length here.[23]

In general, however, sewage disposal falls into one of three categories: (a) dilution, (b) partial treatment and (c) complete treatment. In the first instance the sewage is simply dumped into some body of water where it will not annoy its originators. Here the breakdown and oxidation of the constituents of the sewage occur in nature, and, if sufficient time elapses, no trace remains beyond an increase in nitrate which, in turn, serves as food material for phytoplankton. When this transformation takes place in a flowing stream, the phenomenon is known as the *self-purification of streams*. The essential element, however, is not the fact that the water is in movement but that sufficient time elapses for the breakdown and oxidations to proceed to completion. Soil polluted with sewage similarly "purifies itself."

With increasing population densities the disposal of sewage by dilution becomes unsatisfactory because a body of water is not infrequently a water supply to a neighboring community. Some form of treatment, then, becomes obligatory; in other words, the decomposition is made to take place in whole or in part in the various tanks and other devices making up a sewage treatment plant rather than allowing it to occur in natural bodies of water. In practice, treatment takes the form of preparatory processes followed by a period of anaerobic digestion, then a period of aerobic oxidation. Treatment may be complete even through nitrification, or the partially treated sewage may be disposed of by dilution.

When the disposal of fecal material is an individual or family problem the mechanics involved are somewhat different, but the processes of decomposition and oxidation are essentially the same whether a privy, cesspool or septic tank is used.

It will be noted that, although sewage treatment is basically a bacteriological process, the pathogenic bacteria are not involved; their presence is not taken into consideration, nor is there any effort to destroy them except in the rare instances in which the effluent from a complete treatment plant is chlorinated. In one sense, then, sewage treatment is not directed toward the control of water-borne disease. The fact that it is treated, however, is of considerable significance in this connection. When sewage is disposed of by dilution, the typhoid bacillus and related microorganisms do not multiply; rather, a decline in numbers sets in immediately, and these bacteria do not survive as long in water as in soil, probably not more than a few days or a week. It is probable that they tend to disappear equally rapidly during sewage treatment, although there is no assurance that pathogens are absent in the effluent from treatment plants. Clearly then, fresh pollution is more dangerous than past pollution, and, in a sense, treatment obviates the possibility of such fresh pollution. The economic and esthetic aspects of sewage treatment are, of course, another matter.

[23] See Imhoff and Fair: *Sewage Treatment*. John Wiley & Sons. New York. 1940.

CHAPTER 11

THE BACTERIOLOGY OF MILK AND FOOD

As a vector of infectious disease, milk differs from water in that it is an excellent medium for the growth of many pathogenic bacteria, and from other foods in that it is the only food of animal origin that is consumed in large part in the raw state. Since large quantities of milk are consumed—it is estimated that about 16 per cent of the average dietary in the United States consists of milk and milk products—the importance of this substance in the transmission of disease is evident. The diseases transmitted by milk are: first, diseases of cattle transmissible to man and including bovine tuberculosis, undulant fever, foot-and-mouth disease and streptococcal infections from infected udders; and, second, diseases of man in which the milk serves as the link between man and man, such as typhoid fever, septic sore throat, scarlet fever, diphtheria and, rarely, certain other diseases, such as poliomyelitis.

Sources of Bacteria in Milk.—Unlike water, milk has no native bacterial flora, and it is probable that milk as secreted into the udder of a healthy cow is sterile. The milk in the udder is, however, rarely if ever bacteriologically sterile, for the microorganisms invade the udder via the milk ducts of the teats and the first portion of the milk drawn (fore-milk) always contains more bacteria than the last (strippings). There is, furthermore, no bacterial flora that is characteristic of milk; the presence of microorganisms is always a consequence of contamination and the types of bacteria found are determined by the source of contamination. From this point of view the bacteria of milk fall into two groups; first, those which are present in the tissues of an infected cow and find their way into the udder; and, second, those which enter the milk, usually after it is drawn, from sources external to the animal.

Bacteria from Infected Cattle.—Of these microorganisms perhaps the most important is the *tubercle bacillus*. These bacteria get into the milk directly as a consequence of tuberculosis of the udder, which occurs in 1 to 2 per cent of infected cows, and indirectly by contamination with cow manure when the infectious sputum is swallowed and discharged with the feces. In either case the milk is infective for man, the bovine variety of the tubercle bacillus giving rise, as a rule, to bone and joint rather than pulmonary tuberculosis (p. 576). Milk may, of course, be infected with human tubercle bacilli from infected persons. A high proportion of the former type of infection occurs when the transmission of this disease is allowed to occur. The disease may be controlled at its source, *i.e.*, by the elimination of infected cattle from dairy herds, a practice which is practically universal in the United States.

A bovine variety of *Brucella melitensis*, the causative agent of undulant fever (Chapter 22), infects cattle, producing the disease contagious abortion (the bacterium is sometimes designated *Brucella abortus*). This microorganism is excreted in the milk and infects man, producing a mild type of undulant fever. The caprine variety of this bacterium which is present in the milk of infected goats produces a much more severe disease in man. Undulant fever, like tuberculosis, may be controlled by control of the disease in the animal reservoir of infection.

The virus of foot-and-mouth disease, a virus disease of cattle, is excreted in the milk and is thus transmitted to man. The disease in man is mild, however, and not of great public health importance. More important are the streptococcus infections of the udder, designated garget or mastitis. *The Hotis test* is commonly used for the detection of mastitis; it consists of incubating fresh milk in the presence of 0.025 per cent bromcresol purple for twenty-four hours at 37° C. A positive reaction, the formation of yellow flakes on the side of the test tube, is dependent upon the presence of the streptococci and of agglutinins in the milk; the growing bacteria are clumped by the antibody and the acid reaction results from the fermentation of lactose. The indeterminacy of streptococcus species, coupled with the fact that the same type of streptococcus may produce more than one disease (p. 335), makes it difficult to evaluate the significance of streptococcus mastitis to milk-borne disease. Milk-borne epidemics of septic sore throat, however, are not infrequently associated with acute udder inflammation in the dairy herd and the massive and continuous infection occurring in some of the outbreaks indicates that the udders of the cattle were infected. It has been suggested that the streptococci of scarlet fever may proliferate, with or without symptoms, in the udder, though it is probable that in most instances milk-borne scarlet fever is a consequence of direct human contamination.[1] It may be noted that the udder may be infected occasionally with diphtheria bacilli, which produce small external ulcers, but this is an uncommon occurrence.

Bacteria from External Sources.—When milk is collected under ordinary conditions, the udder bacteria form but an insignificant fraction of the total number of microorganisms in the milk. The skin of the cow, the hands of the milker, the vessels used for collection, and the dust of the cow barn all contribute their quota to the number of bacteria found immediately after milking. If milk is obtained with aseptic precautions, it contains only a few hundred (200 to 400) bacteria per cubic centimeter; collected with somewhat less care, it may contain a few thousand (2000 to 6000); with careless manipulation, even freshly drawn milk may be highly contaminated (30,000 to 100,000 per cubic centimeter). If milk is kept at 0° C. (32° F.), it shows a decrease in the bacterial content during the first few hours, but at higher temperatures the rate of multiplication is high and, when richly seeded at the outset, enormous numbers of bacteria result.

The non-pathogenic bacteria that are present in milk are often differentiated on a physiological basis into the following groups:

[1] For a discussion of streptococcus infections of the cow see Brooks: Jour. Amer. Vet. Med. Assn., 1939, *94*:11.

(1) the acid-forming bacteria,
(2) the alkali-forming bacteria,
(3) the proteolytic bacteria and
(4) the inert bacteria.

The first group includes the fermentative bacteria, and the most common type of fermentation is the lactic acid fermentation, the process by which milk usually sours under natural conditions. A variety of bacteria may be responsible, among them such familiar forms as *Staphylococcus aureus*, *Streptococcus pyogenes* and *Bacterium coli*. A few species, however, are commonly active in the natural souring of milk, and these may be divided into two groups. One of these is comprised of the capsulated gas-forming bacilli of the *Bacterium* (*lactis*) *aerogenes* type, which are closely related to *Bact. coli*, differing principally in their possession of capsules, lack of motility, and ability to produce gas from potato starch. The second type, a streptococcus, *Streptococcus lacticus* (or *lactis*), is abundant in naturally soured milk, particularly when the acidity is high.

Lactic acid milks, regarded by some as having therapeutic value in the treatment of certain intestinal disorders, have been prepared by the inoculation of *Lactobacillus* species such as *acidophilus* and *bulgaricus*. The lactobacilli, however, are commonly found in the fermentation of ensilage and are present in small numbers in the human mouth and intestinal tract and are not ordinarily responsible for the natural souring of milk.

Although lactic acid is commonly the predominating acid in the fermentation of milk, the formation of butyric acid is observed occasionally. This is generally a consequence of the presence of the anaerobic butyric acid bacteria, but may be brought about through the agency of certain aerobes closely allied to *Bacillus subtilis*.

The spontaneous alcoholic fermentation of milk is less usual under natural conditions than either the lactic or the butyric, and the preparation of certain alcoholic beverages is dependent upon the artificial production of this form of milk fermentation. Koumiss, a drink prepared by Tartars by the alcoholic fermentation of mare's milk, and kefir, an effervescent sour milk prepared by inhabitants of the Caucasus from the milk of cows, goats and sheep, are both prepared by the inoculation of fresh milk with old koumiss in the first instance and with "kefir granules" in the second. The bacteriology of the koumiss fermentation is not well known, but in the case of kefir both a bacterium and a yeast appear to be involved. Some species of yeast, it may be noted, are able to effect the alcoholic fermentation of milk in pure culture.

The alkali-forming bacteria are those organisms which do not ferment lactose but, presumably, act upon the nitrogenous substances present with the liberation of ammonia. When typhoid and paratyphoid bacilli, for example, are cultivated in litmus milk no effect is apparent beyond a slowly increasing alkalinity. Certain other bacteria, such as some of the aerobic spore-forming types, also produce lipase and decompose the fats present, converting the milk to a yellow, transparent fluid.

The proteolytic bacteria also produce an alkaline reaction and, in addition, hydrolysis of the milk proteins. Two enzymes, or groups of enzymes, are

responsible; one, a rennin-like enzyme, precipitates the protein with the formation of a soft curd, and a second, casease, brings about hydrolysis of the protein which, when complete, results in the conversion of the milk to a clear fluid, a process sometimes termed peptonization. Peptonization, however, does not follow precipitation when the microorganism does not possess casease. The bacteria producing these changes include spore-forming aerobes such as *Bacillus subtilis*, certain strains of staphylococci, *Proteus vulgaris* and others. Proteolysis may occur in heated milk in which the non-spore-forming lactic fermenters have been destroyed, leaving the more resistant spore-forming proteolytic forms.

The "Diseases" of Milk.—A series of unusual or abnormal changes, sometimes called "diseases" of milk, are produced by certain bacteria which occasionally find their way into milk. "Blue milk" (*Bact. cyanogenes*), "red milk" (*Bact. prodigiosus, Bact. erythrogenes et al.*) and "yellow milk" (*Bact. synxanthus*) are caused by the presence of various chromogenic bacteria. "Bitter milk," characterized by a bitterness that sometimes develops after a short interval, is likewise due to the products of certain microorganisms. Milk sometimes suffers from a ropy or slimy fermentation which, under most circumstances, is regarded as undesirable, although such a fermentation is intentionally produced in the manufacture of Edam cheese in Holland through the action of a particular species of streptococcus.

The *inert bacteria* are those which produce no visible change in milk. These include certain non-pigment-forming bacteria from water and other sources, and, in addition, most of the pathogenic bacteria that find their way into milk. Such dangerous contamination, then, is inapparent without bacteriological examination.

The Pathogenic Bacteria from External Sources.—The multiplicity of sources of contamination results in the heterogeneous bacterial flora that may be found in milk. It is important from the hygienic view that, in addition to microorganisms of soil and water, the bacteria carried by man have relatively ready access to milk. The opportunity for contamination with the bacteria of human disease is, then, ever present, not only when the milk is drawn but throughout its handling until it reaches the ultimate consumer. Contamination may take place directly, as in the case of scarlet fever and other streptococci or diphtheria bacilli from the throat and typhoid bacilli from the hands of infected individuals, or it may be indirect, as when the water used to wash the milk cans is contaminated with typhoid bacilli. In any case, the microorganisms do not simply survive, as in water, but actively multiply and may be present in huge numbers in pooled milk of which but a single part was originally contaminated.

Clearly, then, the most important factors which govern the number of bacteria which may be present in milk are, first, the kind and degree of initial contamination and, second, the temperature at which the milk is kept. The production of hygienically satisfactory milk, therefore, involves cleanliness in the first instance, and immediate cooling and storage at a low temperature in the second. Practical experience has more than adequately proved the significance of these points.

The Bactericidal Property of Fresh Milk.—As indicated above, the

number of bacteria present in freshly drawn milk frequently shows an initial decrease. The extent of this diminution is, perhaps, exaggerated by plate counts; the various antibodies are present in the milk, not only the bactericidal substances but the agglutinins as well, which, by clumping the bacteria, may bring about decreased plate counts. That freshly drawn milk is both bactericidal and bacteriostatic, albeit to a mild degree, is, however, definitely established. This activity is thermolabile, being destroyed in fifteen minutes at 75° C. and in two minutes at 80° to 90° C., and disappears a few hours after the milk is drawn.

MILK-BORNE EPIDEMICS OF DISEASE*

Disease	Outbreaks		Cases	
	Number	Per Cent of Total	Number	Per Cent of Total
Typhoid and paratyphoid fever	76	45.2	1,209	12.1
Septic sore throat and scarlet fever	57	34.0	6,812	68.2
"Gastro-enteritis"	24	14.3	1,423	14.2
Bacillary dysentery	5	3.0	411	4.1
Diphtheria	5	3.0	123	1.2
Poliomyelitis	1	0.6	11	0.1
Totals	168	100.0	9,989	100.0

* In New York State exclusive of New York City as reported by Dublin, Rogers, Perkins and Graves: Amer. Jour. Pub. Health, 1943, *33*:157.

The Determination of the Quality of Milk.—By far the best index of the quality of milk is the number of bacteria it contains. The bacteria present are, in all cases, a result of contamination, and hence these microorganisms are reliable indicators of cleanliness and care and are generally used for this purpose.

Plate Counts.—The total bacterial count of a milk is, then, a reflection of its hygienic quality, and the plate count has been, and still is, widely used in the bacteriological grading of milk. Standardization of the media and of the technique is, of course, necessary for comparable results. A standard procedure has been developed under the auspices of the American Public Health Association, whose publication[2] may be consulted for the details.

Standards of milk quality in terms of plate counts are in general use but vary from one locality to another. In some instances more than one grade of milk is allowable; for example, a "Grade A" milk may contain not more

[2] *Standard Methods for the Examination of Dairy Products.* American Public Health Association. 8th ed., 1941. For a discussion of current practical application of these methods see Black: Pub. Health Rep., 1943, *58*:1605, 1641, 1681.

than 30,000 bacteria per cubic centimeter as delivered, and a "Grade B" milk not more than 100,000. There is a steady movement toward more strict requirements, and the maximum number of bacteria allowed is continually reduced. More than one grade of milk is, however, undesirable in that the distribution of inferior milk is allowed, and, when possible, a single standard is preferable. In Chicago, for instance, there is but one grade of milk, and it must not contain more than 10,000 bacteria per cubic centimeter as delivered to the consumer.

Microscopic Counts.—Besides the ordinary macroscopic colony count obtained by plating, a microscopic method devised by Breed[3] is of value for many purposes. In the Breed method milk is taken in a capillary pipette discharging 0.01 cc. and is dried over an area of one square centimeter on a glass slide. After washing out the fat with xylol and fixing with alcohol, the film is stained with methylene blue. The number of bacteria per square centimeter is estimated by counting a carefully measured area. The ratio used in comparing the microscopic count with the standard plate count is 4 : 1, although it is recognized as inaccurate and variable. This direct microscopic count does not involve incubation and has proved of special value in judging the quality of fresh milk as delivered to milk-receiving stations.

Methylene Blue Reduction.—If a small amount of methylene blue is added to milk and the mixture incubated, the dye will, in time, be reduced to the colorless leuco base. Although freshly drawn milk has some small power to reduce this and other dyes, the reduction is, for all practical purposes, a consequence of the metabolic activities of contained bacteria. There is, then, a direct relation between the length of time required for reduction and the number of bacteria present, and the measurement of this reduction time provides a useful approximation of the quality of a milk. The method is best adapted to raw milk and has the great advantage that no special apparatus or training of the operator is required. Although milk cannot be graded with any degree of precision in this way, it is generally agreed that a milk decolorizing in less than two hours is poor in quality, while that which does not decolorize in eight hours is excellent. Resazurin has been used to some extent in the place of methylene blue.

Bacterium Coli Count.—It might be assumed that since *Bact. coli* may be used as an index of the sanitary quality of water, it could occupy a similar position with regard to milk. It will be recalled, however, that water contamination of sanitary significance is fecal, whereas this is not the case with milk; *Bact. coli*, for example, would not be associated with the presence of the diphtheria bacillus in milk. Furthermore, since the great majority of market milks contain cow feces, the estimates varying from 60 to 100 per cent, *Bact. coli* in milk is probably most often of bovine origin. Although the *coli* count has been used widely in the past, the differentiation of these microorganisms is not worth while except under special circumstances that warrant it.

The Isolation of Pathogenic Bacteria.—In contrast to water, the isolation of pathogenic bacteria from milk is not only a practical but often a desir-

[3] Breed: N. Y. State Agr. Exp. Sta. Bull. No. 443, 1917.

able procedure. The presence of tubercle bacilli, for example, can be conclusively shown only through the isolation of these microorganisms, in this instance usually by guinea-pig inoculation. Other pathogens, such as hemolytic streptococci, *Brucella*, etc., may be isolated by culture. The isolation of these bacteria is not, of course, a routine procedure but is frequently carried out when an outbreak of disease is suspected of being milk-borne. The details of the methods employed may be found elsewhere.[2]

Cell Count and Sediment Test.—The number of leucocytes present in a sample of milk and the amount of contained dirt that can be strained out through a standard cotton disc are frequently of value in the assay of milk quality. Excessive numbers of leucocytes are present in the milk from infected udders, and when their presence is noted in Breed smears, mastitis is suggested. The amount of sediment that a sample of milk contains is, of course, an index of the extent to which it has been contaminated and is often, though not necessarily always, correlated with the bacterial count.

The Hygienic Control of Milk.—The sanitary quality of milk may be controlled in one or both of two ways: in the first instance, by preventing to a considerable degree the contamination of milk and the multiplication of contained bacteria; and, in the second, by destruction of bacteria, the pathogenic forms in particular, already present in the milk.

Inspection.—The periodic inspection of dairy farms is carried out by the local board of health in many places, and, although it does not insure the absence of pathogenic bacteria from milk, it is effective in increasing cleanliness and reducing the numbers of bacteria. A score card is frequently used, and a given dairy farm is rated according to a system of points.

Certified Milk.—One of the earliest attempts to avoid the dangers of milk-borne infection was the elaboration of methods designed to safeguard milk at every step in its production, collection and distribution. To this end "Medical Milk Commissions" were established in a number of localities in the United States, usually under the auspices of the local medical society. Milk conforming to certain standards is certified by such a commission to be of high quality. The regulations, which are generally excellent, deal with such matters as the cleanliness of barnyard and dairy; the purity of the farm water supply; the proper sterilization of utensils; and the health of the cows and of the milkers. The bacterial content is limited to 10,000 per cubic centimeter and the milk must be delivered within thirty-six hours.

Certified milk is undoubtedly safer to use than milk collected and transported without suitable supervision, and the work of the milk commissions has done much to improve dairy conditions in many parts of the country. At the same time, raw milk, certified or not, can never be regarded as protected against all chances of contamination; the difficulty—not to say impossibility—of making sure that no typhoid carriers and no persons suffering from a mild case of diphtheria or scarlet fever are ever employed in a dairy is, of course, self-evident. Outbreaks of diphtheria, paratyphoid fever and other diseases have, in fact, been traced to certified milk. For this reason, as well as because of the relatively high cost of production, the use of certified milk remains limited.

Pasteurization.—The process of destroying pathogenic bacteria in milk is by far the most satisfactory method of controlling milk-borne infection. The use of a temperature high enough to kill most microorganisms but not so high as to produce radical alterations in the substance heated was first applied by Pasteur to preserving wines without destroying their original flavor or bouquet. Although still widely used in connection with bottled beer and wines, the process of pasteurization is now used chiefly for the treatment of milk.

The temperature to which milk is raised and the time for which it is held there are, of course, dictated by the heat resistance of the bacterium to be killed. From the beginning attention has been directed primarily toward the tubercle bacillus, and it has been found that this bacterium is killed by exposure to a temperature of 140° F. for twenty minutes. In practice, then, a temperature of 142° to 145° F. for a period of thirty minutes provides an adequate margin of safety, and these are the requirements that are usually specified.[4] It should be noted that the technical aspects of such treatment of milk on a large scale are of prime importance, taking the form of prevention of foaming, proper design of valves to prevent "cool pockets" and dead ends, and the like. It might be supposed that a higher temperature or longer holding time resulting in a greater margin of safety would be desirable; this is, however, not the case, for if either the time or the temperature is increased alterations take place in the physical state of the milk in which the fat is dispersed into smaller globules and will not rise to the top on standing. Such a disturbance of the "cream line" is, of course, of esthetic rather than sanitary significance.

Considerably higher temperatures and a shorter holding time have been and still are used to a limited extent. In the so-called "flash" process the temperature is raised to 160° F. and maintained for fifteen seconds, then the milk is immediately chilled, as it is in the "holding" process discussed above. Both time and temperature are difficult to control accurately in the flash process, and this fact, together with the "cooked" taste imparted to the milk, has severely limited the use of this method.

The plate count of milk is tremendously reduced by pasteurization, for not only are the pathogenic bacteria, such as the tubercle bacillus, *Brucella abortus*, streptococci and the like, destroyed, but the majority of other bacteria present as vegetative cells are killed also. The efficiency of the process may, then, be measured in terms of bacterial destruction and, in the last analysis, must be measured this way. Very recently, however, a test has been devised which apparently gives an accurate measure of the efficiency of pasteurization as carried out in practice. The *phosphatase test* is based upon the presence of the heat-sensitive enzyme phosphatase in milk. Since 96 per cent of the enyzme is destroyed by heating to 143° F. for thirty minutes, the amount remaining in a pasteurized milk may be used as an indicator of the pasteurization as carried out. The enzyme liberates phenol from phosphoric-phenyl esters and, as originally proposed, the test consisted of the addition of disodium phenol phosphate and Folin's reagent, incubating eighteen to twenty-four hours, and reading the blue

[4] Amer. Jour. Hyg., 1927, *7*:147.

color developed. A variety of modifications have, however, been proposed, and as yet the test has not been standardized,[5] though it has become increasingly widely used.[6]

Not all bacteria are killed by pasteurization. Besides the resistant spore formers, aerobic and anaerobic, certain streptococci are able to survive the pasteurizing temperature. These are the lactic acid formers rather than the pathogenic forms, and pasteurized milk sours in the ordinary way on standing, though a longer period of time is required—*i.e.*, its keeping qualities are improved. If higher temperatures are used, 180° F., the lactic acid bacteria are killed and proteolysis occurs as the milk spoils. Thermophilic bacteria are frequently present in pasteurized milk in great numbers, for the temperature of pasteurization is an incubation temperature for these microorganisms. The slight acid metallic taste occasionally noticeable in pasteurized milk is often attributable to their biochemical activity.

It must be emphasized that the process of pasteurization, while effective in destroying pathogenic bacteria present in milk and, incidentally, increasing its keeping qualities, is not to be regarded as an excuse for the marketing of dirty and highly contaminated milk. There is some evidence, for example, that the growth of enormous numbers of bacteria, albeit nonpathogens in the usual sense, is associated with summer diarrhea of infants. In most cases, therefore, sanitary regulations specify not only the allowable number of bacteria in pasteurized milk as delivered but also an upper limit for raw milk which is to be pasteurized.

The Regulation of Milk Quality.—The application of appropriate methods of rendering and keeping milk satisfactory from the hygienic point of view is, essentially, a social and legal problem rather than a scientific one. To this end appropriate ordinances are more and more generally incorporated into the legal structure of the community, and these, when adequately enforced, produce a marked reduction in milk-borne disease. A standard form of ordinance developed by the United States Public Health Service[7] had in 1938 been adopted by more than 800 American cities. In any case, the grading and pasteurizing of milk has become general in the United States. A recent survey[8] has shown that about half the cities of over 1000 population grade milk and permit the sale of one grade of raw milk and one grade of pasteurized milk; of the total volume of market milk about 74 per cent is pasteurized, 99.4 per cent is from tuberculin-tested herds, and 35 per cent from abortion-tested herds. In general, sanitary regulations are somewhat more strict and more rigorously enforced in the large cities. In Chicago, for example, all milk is pasteurized, including certified milk, and this regulation is rigidly enforced.

Such practice has resulted in marked reductions in the number of bacteria in market milk. In 1901 the bacterial content of market milk in New York City varied from 300,000 in the coldest weather to 5,000,000 during

[5] For discussion and references see the appendix of *Standard Methods for the Examination of Dairy Products, loc. cit.*

[6] Burgwald: Jour. Dairy Sci., 1942, *25*:285.

[7] Pub. Health Repts., 1926, *41*:1604.

[8] Fuchs and Frank: Pub. Health Bull. No. 245, 1938; also Pub. Health Repts., 1942, *57*:228.

the summer months; in Chicago (1904) the counts ranged from 10,000 to 74,000,000, and in Boston (1892) averaged 4,500,000. The incidence of milk-borne disease has correspondingly decreased; for example, in 1907–1915 there were in Massachusetts 2215 cases of typhoid fever which were traced to milk, but in 1919–23 only 297.

Milk Products.—Various foods made from milk, such as ice cream, butter, cheese and the like, are potential vectors of disease when made from milk contaminated with pathogenic bacteria. The bacteria tend to die out upon storage, of course, although it has been found that the typhoid bacillus will survive for three months or more in butter,[9] and tubercle bacilli have been found in butter and certain quick-ripening varieties of cheese. Ice cream may serve as a vector for typhoid fever, scarlet fever and the like. Pasteurization of the mix is customary and generally at higher temperatures than those used for milk. Human infections with foot-and-mouth disease have been traced to contaminated butter and cheese, but the public health significance of these findings is problematical.

FOOD POISONING AND FOOD-BORNE INFECTION[10]

The diseases transmitted by milk and milk products may be disseminated by a variety of other foods; in addition to these, however, other types of illness may result from the ingestion of contaminated foods which make up that group of affections designated as food poisoning. The kinds of illness that may result from the ingestion of food may be summarized briefly:

(1) individual idiosyncrasies;
(2) toxemia from foods, such as
 (a) foods naturally poisonous,
 (b) foods into which poisons have been accidentally introduced and
 (c) foods containing poisons of bacterial origin formed by
  1. *Clostridium botulinum* and
  2. staphylococci;
(3) food-borne infection, including both
 (a) bacterial infections, such as
  1. typhoid fever, dysentery, cholera *et al.* and
  2. *Salmonella* infection, and
 (b) parasitic infections.

It will be clear that in some instances food serves simply as a vector in the transmission of diseases such as the protozoan infections and the enteric diseases. In the remainder, however, the clinical manifestations are those associated with food poisoning proper—vomiting, diarrhea, enteritis and a greater or lesser degree of prostration. Although a number of types of food poisoning given above are not bacterial in origin, their clinical symptoms frequently are those of bacterial food poisoning, and these must be considered in any attempt to ascertain the etiology of a given outbreak, even

[9] Berry: Jour. Prev. Med., 1927, *1*:429.

[10] Jordan: *Food Poisoning and Food-Borne Infection.* University of Chicago Press. Chicago. 2nd ed., 1931. Dack: *Food Poisoning.* University of Chicago Press. Chicago. 1943.

though food poisoning is, in a majority of instances, a consequence of bacterial activity. Hypersensitivity (p. 301) to a given food substance, for example, is frequently manifested as vomiting, and an outbreak confined to a family may be the result of familial tendency; similarly, the gastro-intestinal disturbances following the ingestion of naturally poisonous foods such as toadstools, or foods contaminated with poisons such as arsenic or cyanide, are often indistinguishable from those induced by some poisons of bacterial origin.

Food Poisons of Bacterial Origin.—Often popularly termed "ptomaine posioning," poisoning with food containing toxic substances of bacterial origin is very common, probably more so than is generally recognized. The term "ptomaine poisoning" is a misnomer that is both misleading and inaccurate. The organic bases such as putrescine, cadaverine, methylamine and the like, which have been called ptomaines and which result from the bacterial decomposition of protein, are not toxic when given by mouth. Neither are other decomposition products toxic *per os*. While a partially decomposed food may be esthetically unattractive, the innocuous nature of the products of decomposition is obvious when one considers the advanced state of decomposition reached by some cheeses. Toxicity is, on the contrary, attributable to the presence of substances synthesized by the bacteria whose presence may or may not be associated with obvious evidence of decomposition of the food substance.

Botulism.—Of the toxic substances formed by bacteria in food the most powerful is the toxin of *Clostridium botulinum* (p. 536). In the United States canned foods provide the anaerobic conditions necessary for the germination of botulinus spores which have survived processing, while in Europe meat products, particularly the larger sausages, have been more frequently involved. The microorganism never infects man under natural conditions, and the disease is an intoxication resulting from the ingestion of preformed toxin present in the food substance. The disease is not of great public health importance—about 2000 cases have been reported in all—and the outbreaks are generally limited in scope, often being confined to a family circle of a few individuals. The case fatality is high, however, probably 60 to 80 per cent, and when outbreaks do occur they attract considerable attention. This type of food poisoning differs from the others in that the symptoms are not necessarily gastro-intestinal; the toxin acts upon the peripheral nerves and the symptoms are those resulting from such damage. The use of botulinum antitoxin is discussed elsewhere.

Since both anaerobic conditions and a period of incubation are required for the growth of *Cl. botulinum* and the formation of toxin, fresh foods are not involved in this type of food poisoning. The toxin is destroyed by heat, hence freshly cooked foods do not contain it. Canned foods and certain meat products which are consumed either cold or after simply warming may contain botulinum toxin if the spores of this bacterium were originally present and not destroyed. Commercial processing as now employed in this country is adequate to destroy botulinum spores, and at the present time outbreaks of botulism are frequently attributable to the consumption of home canned, insufficiently heated foods. The presence of toxin is not

necessarily associated with obvious signs of spoilage, though it is probable that toxin-containing foods are never entirely normal in appearance, odor and the like.

Staphylococcus Food Poisoning.—The marked gastro-intestinal disturbances characteristic of food poisoning are not infrequently associated with the consumption of foods containing starch thickening, such as eclairs, cream puffs, certain types of cake fillings and salad dressings, and the like. Upon bacteriological examination, the incriminated food generally is found to contain enormous numbers of bacteria, sometimes staphylococci, other times bacteria of the colon-aerogenes group, streptococci, *Proteus* and similar microorganisms.

The causal relation of staphylococci, generally, though not necessarily, hemolytic strains of the *Staphylococcus aureus* group, to food poisoning was suggested in 1914[11] and in recent years this relationship has been definitely established.[12] These bacteria produce a soluble toxic substance, or *enterotoxin*, which gives rise to typical food poisoning symptoms in man and *rhesus* monkeys upon feeding and in kittens upon injection. There is considerable doubt as to the specificity of the kitten test, however, for it has been shown that the staphylolysins will cause vomiting on injection into these animals.[13] The incubation period in man is short—two to six hours—and the case fatality *nil*, with complete recovery in twenty-four to forty-eight hours. The nature of the enterotoxic substance is unknown, although its formation by staphylococci appears to be favored by the presence of starch. A period of incubation is necessary for its elaboration by the bacteria, and in outbreaks of this type of food poisoning it is always found that a period of time, generally not less than eight hours, has elapsed between the preparation and the consumption of the food. This type of food poisoning is quite common and a number of well authenticated outbreaks have been reported.[14]

Whether or not bacteria other than the staphylococci form similar enterotoxic substances is open to question. The fact that the same incubation period and clinical symptoms are observed when epidemiological evidence incriminates the other bacteria noted above suggests that enterotoxin may be formed by them. Streptococci are occasionally incriminated in food poisoning outbreaks with evidence of enterotoxin formation,[15] and *Bacterium aerogenes*, *Proteus* and similar organisms have been found under much the same circumstances. There is some experimental evidence[16] indicating that the ability to form substances irritating to the human alimentary tract may be one that is possessed by a variety of bacteria, but as yet this is not definitely established.

Food-Borne Bacterial Infections.—The food-borne bacterial infections are of two general types. The one consists of those diseases which are

[11] Barber: Philippine Jour. Sci., 1914, *9*:515.

[12] Dack, Cary, Woolpert and Wiggers: Jour. Prev. Med., 1930, *4*:167; Jordan: Jour. Amer. Med. Assn., 1930, *94*:1648.

[13] Fulton: Brit. Jour. Exp. Path., 1943, *24*:65.

[14] *Cf.* Jordan and Burrows: Amer. Jour. Hyg., 1934, *20*:604.

[15] Foley, Wheeler and Getting: Amer. Jour. Hyg., 1943, *38*:250.

[16] Jordan and Burrows: Jour. Inf. Dis., 1935, *57*:121.

transmitted by a variety of vectors of which food is but one and whose clinical symptoms are not those usually associated with food poisoning. Such, for example, are typhoid and paratyphoid fevers, dysentery, cholera and other enteric infections. The second type of infection is that with bacteria of the *Salmonella* group, in which the incubation period is short, the gastro-intestinal disturbance is of short duration, a day or two, and the symptoms are typical of food poisoning. There is some crossing between the two types, for paratyphoid B bacilli (*Salmonella paratyphi B*) may produce either the typical food poisoning response or paratyphoid fever.

Food-borne typhoid, dysentery and other diseases of the first group ordinarily occur on a small scale; the great epidemics are water-borne and, to a lesser extent, milk-borne. Although limited in scope, food-borne infection plays an important part in maintaining some of these diseases in endemic form. The so-called "residual typhoid," for example, which remains in spite of hygienic control of water and milk supplies is, in large part, food-borne. The employment of cooks and other food handlers who are typhoid carriers provides the opportunity for food infection and consequent transmission of the disease. Mary Mallon—"Typhoid Mary"—was one of the most notorious examples of a cook who was a typhoid carrier.

Of the *Salmonella* infections, one of three species is generally involved, *Salmonella typhi-murium* (*S. aertrycke*) and its varieties (*Newport, Stanley, etc.*), *Salmonella enteritidis* and *Salmonella cholerae-suis* (including Voldagsen and *paratyphi C*). *S. typhi-murium* is the most frequently observed, while *S. cholerae-suis* is only rarely present. The ingestion of food containing large numbers of these organisms frequently results in the typical symptoms of food poisoning. No enterotoxic substance has been shown to be formed by these bacteria, and it is probable that actual infection takes place as indicated by the somewhat longer incubation period and the finding of the bacteria in the feces. The case fatality is variable, ranging from zero to 10 per cent. Almost any kind of food may serve to carry these microorganisms, although meats and other protein foods predominate. This type of food poisoning is not common.[17]

Both *S. typhi-murium* and *S. enteritidis* are commonly carried by rats and mice, and it is probable that in many cases these rodents are responsible for the infection of food. In others the source of infection may be a human carrier, and in many cases of meat poisoning it has been found that the meat was from a diseased animal.

The food-borne parasitic infections include the various flukes, tapeworms, echinococcus and round worms that infect man. In these diseases the infective stage of the parasite is often present in a food substance as a consequence of its life cycle and the mechanisms involved may be found elsewhere. (See Chapter 34.)

[17] Savage and White: *Food Poisoning: a Study of 100 Recent Outbreaks.* Medical Research Council Special Report Series No. 92, 1925.

CHAPTER 12

IMMUNITY—ANTIGENS, ANTIBODIES AND THE ANTIGEN-ANTIBODY REACTION[1]

It has been common knowledge for many years that recovery from certain of the infectious diseases is accompanied by the development of an enhanced resistance, and second attacks of a disease once overcome are not common. This enhanced resistance, or immunity, is specific, *i.e.*, an individual immune to one disease may be no more than ordinarily resistant to others, and is variable from one disease to another, some giving rise to a "solid" immunity of long duration while in others the immunity is imperfect or partial and transient. The specific immune state supplements the complex of factors which make up non-specific resistance to infection, and in some instances even a solid immunity may be broken down by fatigue, malnutrition and similar factors which are not consistent with a state of physiological well-being. Immunity arises as a consequence of the reaction of the host to intimate contact with the parasite, or its products, within the tissues, and constitutes a last line of defense whose presence frequently prevents infection and whose development during an attack of disease determines the outcome. The reaction of the host to the invading microorganism has been studied intensively since the early days of bacteriology, and the body of knowledge so accumulated makes up the extraordinarily complex science of immunology. These studies have not only led to some degree of understanding of the phenomenon of specific resistance to infection but in addition have provided biology with a new method whose general application has been, thus far, limited.

ANTIGENS

An antigen is ordinarily but unsatisfactorily defined as any substance whose introduction into the tissues of an animal results in the appearance, after a suitable length of time, of antibodies in the blood serum and other body fluids. With certain exceptions, the reaction is *specific* in that each antigen stimulates the formation of antibody for itself and no other anti-

[1] Much of the earlier work on immunity and related problems will be found in the following volumes: Ehrlich: *Gesammelte Arbeiten zur Immunitätsforschung.* Berlin. 1904; Metchnikoff: *Leçons sur l'Inflammation.* Paris. 1892; *idem., L'Immunité dans les Maladies Infectieuses.* Paris. 1901; Bordet: *L'Immunité.* Paris. 1920. The following modern treatises are of particular value: Zinsser, Enders and Fothergill: *Immunity. Principles and Application in Medicine and Public Health.* The Macmillan Company. New York. 5th ed., 1939; Wells: *Chemical Aspects of Immunity.* Chemical Catalog Co. New York. 2nd ed., 1929; Topley: *An Outline of Immunity.* William Wood & Company. Baltimore. 1933; Medical Research Council (Great Britain): *A System of Bacteriology.* Vol. 6; Marrack: *The Chemistry of Antigens and Antibodies,* Medical Research Council, Special Report Series No. 230, 1939. Boyd: *Fundamentals of Immunology,* Interscience Publishers. New York. 1943.

gen, and it takes place only when the antigen is a *foreign substance* to the animal into which it is injected. This characterization is, of course, one that is based on what antigens do rather than what they are, for knowledge of the nature of these substances is as yet too fragmentary to permit a general definition in terms of composition and configuration of the chemical compounds which exhibit the property of antigenicity.

In general, antigenic substances are proteins, and probably all naturally occurring proteins soluble in the body fluids and containing a full complement of amino acids (the so-called complete proteins) may function as antigens. According to Wells[2] the presence of aromatic radicals is associated with antigenicity; proteins deficient in aliphatic amino acids but containing aromatic amino acids, such as zein, gliadin, egg albumin and casein, are antigenic, while gelatin and protamines which are deficient in aromatic radicals are not antigenic. The property of antigenicity is not destroyed by heating except as the protein is rendered insoluble by coagulation (specificity is somewhat altered), but it is lost upon hydrolysis, probably at a very early stage, although the precise point at which antigenicity disappears during hydrolytic cleavage of the molecule is not known. Antigenicity, then, appears to be a property of the intact, or nearly intact, protein molecule, and there is reason to believe that one prerequisite of antigenicity is a large molecule which may exist in colloidal solution.

Iso-antigens.—It is not strictly true that antibody response is induced only by antigens foreign to the inoculated animal unless the term foreign is taken to mean not present in the circulation of that animal. It was early observed by Hektoen[3] that thyroglobulin would act as an antigen in the same species of animal from which the tissue was taken, and since then it has been found that lactating goats will produce antibody to their own casein and that lens protein is antigenic even in the same individual animal. It is definitely established, therefore, that an animal will produce antibody against certain antigens occurring in its own tissues. Such antigens are designated iso-antigens and their corresponding antibodies iso-antibodies. The organ specificity of certain types of antigens (see below) is probably a related phenomenon. In general, iso-antibody titers are low.

The Human Blood Groups.—It was discovered by Landsteiner[4] that human blood could be divided into four immunological groups which represent the four possible combinations of two antigens present in the erythrocyte. The serum contains antibody (agglutinin) for the absent antigens; antigen and corresponding antibody do not coexist in the same blood. There are three systems of nomenclature for these groups (see table) of which the International is by far the most widely used. Additional immunological factors are also known to be present and are responsible for a part of the observed transfusion incompatibilities; these can be avoided if donor and recipient blood are tested against one another as well as typed. These blood groups are inherited by Mendelian law and for this reason are of

[2] Wells: *The Chemical Aspects of Immunity.* Chemical Catalog Co. New York. 2nd ed.. 1929.

[3] *Cf.* Lewis: Jour. Inf. Dis., 1934, *55*:168.

[4] Landsteiner: Wien. Klin. Wchschr., 1901, *14*:1132.

considerable interest in connection with the question of normal antibodies (p. 296) as well as having some forensic utility in cases of disputed paternity, etc.

The Specificity of Antigens.—Closely associated with the nature of antigens is the specificity of the immunological reactions. Not only does a given antigen stimulate the formation of a specific antibody but it will react, either *in vivo* or *in vitro*, only with its own antibody or antibodies to closely related antigens. For example, the serum proteins of the higher animals, while highly specific for species, show cross reactions with the antibodies to the serum proteins of closely related species; human blood serum shows no immunological relationship to horse or rabbit serum but reacts to some degree with antisera prepared against the serum proteins of anthropoid apes and certain monkeys.[5] Immunological differences between individuals of a single species are, of course, found in the human blood groups. The inheritance of blood groups and the correspondence of immunological relationships of species with generally accepted zoological classifications are indicative of the fundamental significance of antigenic specificity.[6]

HUMAN BLOOD GROUPS

Groups			Erythrocyte antigens	Serum antibodies
International	Moss	Jansky		
O	IV	I	....	a, b
A	II	II	A	b
B	III	III	B	a
AB	I	IV	A, B	....

It should be noted, however, that, although the great majority of antigenic substances are species specific, certain antigens are found to occur in distantly related organisms. Of these the more important are lens protein (actually two proteins, α and β crystallin), which is common to a wide variety of animals, and an antigen known as Forssman antigen, heterophile antigen or, less frequently, heterogenetic antigen. Heterophile antigen has been found in the organs of the guinea pig, horse, cat, dog, mouse, chicken, turtle and several species of fish, in some bacteria, such as certain of the paratyphoid and dysentery bacilli and pneumococci, and in some varieties of maize.[7] It is not present in the erythrocytes of any of these animals but is present in the red cells of the sheep, whose organs do not contain it. It is not found in other organisms such as the pig, ox, rabbit, goose, frog, eel,

[5] Such immunological relationships are discussed at length by Nuttall: *Blood Immunity and Blood Relationship*. Cambridge. 1904.

[6] See the review by Boyden: Physiol. Zool., 1942, *15*:109.

[7] Hyde, Chapman and Kiesling: Amer. Jour. Hyg., 1934, *20*:465.

man, pigeon and rat. This peculiar distribution among species has not been explained.

Still other antigens which are common to, or closely related in, widely different species are those which confer organ specificity. Possibly the immunologically related caseins may be considered in this category but, further, antigens present in a given organ such as kidney are similar to those in the same organ of a different species. These have been studied in some detail by Witebsky.[8] The so-called heavy proteins, isolated from disintegrated normal tissue by ultracentrifugation and thought by some to represent mitochondria, have been of interest in connection with the isolation of similar material from virus-infected tissues which is believed to represent the infectious agent. Immunological studies on these substances by Furth and Kabat[9] and by Henle, Chambers and Groupe,[10] have indicated that heavy proteins from various organs show three kinds of immunological specificity, namely, species specificity or immunological relation to other organs of the same animal, organ specificity, or immunological relation to the same organ of other species, and, finally, specificity for the organ of the one species. Similarly, Bailey and his co-workers[11] have reported finding organ specificity in nutrient media which was derived from the tissues used for the preparation of the infusion. It has been reported by some that organ-specific antisera are specifically toxic for that organ, *i.e.*, nephrotoxic, etc., but the evidence is not altogether unequivocal.

A number of antigens are common to different species of bacteria or to bacteria and certain other organisms and will be discussed in the following chapter in connection with natural immunity.

The Chemical Basis of Specificity.[12]—A large body of sound experimental evidence has established the fact that the specificity of antigens is determined by their chemical composition. Early experimental investigation of a wide variety of antigenic proteins has shown that immunologically identical proteins are, so far as can be determined, identical in composition; that antigenic proteins differing from one another in composition are also immunologically distinct; and that antigens showing some degree of cross reaction are closely related in chemical structure. Conclusive evidence that immunological specificity is a property of certain atomic and molecular arrangements has, however, been obtained through the study of altered specificity and artificial antigens.

The specificity of an antigenic protein may be altered by heating, partial denaturation, treatment with formaldehyde, etc., in such a way that part of the original specificity is lost but species specificity remains although somewhat broadened. Heated rabbit serum, for instance, behaves as a foreign protein in the rabbit, and the antibodies produced will not react with unheated rabbit serum. Retention of species specificity is indicated

[8] Witebsky: Ztschr. f. Immunitätsforsch, 1929, *62*:3.

[9] Furth and Kabat: Jour. Exp. Med., 1941, *74*:247.

[10] Henle, Chambers and Groupe: Jour. Exp. Med., 1941, *74*:495.

[11] Bailey and Gardner: Jour. Immunol., 1940, *39*:543; Bailey and Raffel: Amer. Jour. Hyg., 1941, Sec. B, *33*:86; *ibid.*, 1941, Sec. B, *34*:8; Jour. Exp. Med., 1941, *73*:617.

[12] This subject is discussed in detail by Wells (*loc. cit.*) and by Landsteiner: *The Specificity of Serological Reactions.* Charles C Thomas. Springfield, Ill. 1936.

by the fact that antibodies to the heated serum react with the homologous antigen and to only a slight degree with heated sera from other animals. Such heated proteins are known as *coctoproteins*. Treatment of a protein with iodine, nitric acid or nitrous acid (the last to give the intermediate diazo compounds), on the other hand, alters the specificity of the antigen so profoundly that species specificity is largely destroyed. Nitrated rabbit serum, for example, acts as a foreign protein to the rabbit, and the antibodies developed will react not only with the homologous antigen but with nitrated protein derived from other animal species or even from plants. The general question of loss of species specificity by treatment of this kind has been reviewed by Henry.[13] Since iodine, nitrate and similar substances react with the protein by substitution in the benzene rings of the aromatic amino acids, Pick[14] has interpreted these findings as indicating that species specificity is associated with the aromatic radicals and their arrangement within the protein molecule, a conclusion which, as will appear, is no longer justified.

Largely through the work of Landsteiner and his colleagues[12] in the preparation and study of a large series of artificial antigens, some aspects of the phenomenon of immunological specificity are now relatively well understood. It has been found that the species specificity of antigens may be altered in ways other than attacking the aromatic radicals; acetylation with acetic anhydride, esterification with ethyl alcohol and methylation with diazomethane, all changes in the salt-forming groups of the protein molecule, result in a change in specificity similar to that obtained by treatment with iodine or nitric acid. It is of particular interest that such a marked effect on specificity may be produced by the addition of the small acetyl, ethyl or methyl groups to the large protein molecule. Even phosphorylation has been found[15] to alter the immunological properties of egg albumin.

The influence of the addition of relatively simple organic radicals on the immunological specificity of antigenic proteins has been further elucidated through the study of the immunologic behavior of a variety of compounds prepared from protein and the diazonium derivatives of a number of methyl, chlor, brom and nitro substitution products of aniline and *o*-, *m*- and *p*-aminobenzenesulfonic acid as well as the parent compounds, and others such as *p*-aminophenylarsenic acid, *o*-, *m*- and *p*-aminocinnamic acid, etc. From these and other experiments it is apparent that *the immunological behavior of antigens is specifically modified by a relatively small part of the large protein molecule, and the specificity of the antigen is determined by the chemical structure of this part, cross reactions occurring between antigens whose determinant portions are closely related aromatic groups.* The spatial arrangement in the determinative groups, as well as their nature, is reflected in immunological behavior; the position, *ortho*, *meta*, or *para*, of substituted acid groups in the aromatic radical produces differences in specificity, the stereoisomers of tartaric acid and *p*-aminobenzoylphenyl-

[13] Henry: Jour. Exp. Med., 1942, *76*:451.
[14] Pick: Kolle und Wassermann, *Handbuch d. path. Mikroorganismen*, 1912, *1*:685.
[15] Heidelberger, Davis and Treffers: Jour. Amer. Chem. Soc., 1941, *63*:498.

acetic acid yield immunologically distinct antigens when coupled with protein, and the position of amino acids in peptide-azoproteins is a factor in determining immunological specificity. If it be assumed that the amino acids comprising the peptide chains of the protein molecule are arranged in regular and recurring order and proportion and, consistent with present theory of protein structure, that these chains are folded, then it is apparent that the recurring combinations of amino acids in the folded chains will result in "nodes" of molecular structure. It is supposed that this type of molecular structure confers immunological specificity on the protein molecule that is not combined with some other type of structure.

Haptenes.—The relatively simple substances which, when linked to antigenic protein, determine the immunological specificity of the complex, have been termed *haptenes* by Landsteiner. They are also known as *partial antigens* since, although in the absence of a protein carrier they will not stimulate the formation of antibody, they will react with antibody produced by the haptene-protein complex and, therefore, act as "antigens" in the test tube.

The importance of haptene-determined specificity to the understanding of the immunology of bacteria and the infectious diseases is very great indeed, for it has become increasingly apparent that much of the antigenic material present in the bacterial cell is in the form of haptene-protein complexes. It was found by Zinsser and Parker[16] in 1923 that certain materials found in bacteria and presumably nonprotein in nature, although not capable of stimulating the formation of antibodies, would react with antisera *in vitro*, and these they designated as *residue antigens*. Similar substances have since been found by other workers in a wide variety of bacteria, and investigation has indicated that in many instances these substances function as haptenes, lending immunological specificity to the microorganisms containing them.

Polysaccharides.—Of the residue antigens those of the pneumococcus have been the most intensively studied and for present purposes may be regarded as typical. These so-called *specific soluble substances* (SSS), which make up the capsule of the bacterium, have been found by Heidelberger, Avery, Goebel and others[17] to be polysaccharide in nature. Of the pneumococcal type specific polysaccharides, that of Type III has been the most thoroughly investigated and appears to be a polymer of aldobionic acid units (in this case glucose and glucuronic acid joined through a glucoside linkage, 4-β-glucuronosidoglucose, designated as cellobiuronic acid) having a molecular weight of 1000 to 5600. The nature of the other type specific polysaccharides is not so clear. Type II polysaccharide hydrolyzes to glucose; Type I is possibly a trisaccharide made up of two uronic acid (in part galacturonic acid) molecules, a third molecule containing nitrogen,[18] and acetyl groups whose presence is of considerable immunological significance;

[16] Zinsser and Parker: Jour. Exp. Med., 1923, *37*:275.

[17] *Cf.* Heidelberger: Chem. Rev., 1926–7, *3*:403; Physiol. Rev., 1927, *7*:107; Medicine, 1933, *12*:279; Harvey Lectures, 1933, *28*:184. See also Marrack: *The Chemistry of Antigens and Antibodies*. Medical Research Council Special Report Series No. 230, 2nd ed., 1939.

[18] Heidelberger, Kendall and Scherp: Jour. Exp. Med., 1936, *64*:559.

and Type IV yields an amino sugar on hydrolysis. That such polysaccharide haptenes are not peculiar to the pneumococcus is indicated by the isolation of similar substances from a variety of microorganisms including yeasts and other fungi and certain metazoan parasites as well as many species of bacteria.

The presence of capsular polysaccharide material confers immunological specificity upon the pneumococcus types; the rough, nonencapsulated forms are species specific and no longer may be differentiated into types irrespective of the immunological type of the smooth, encapsulated parent form. The species specificity manifested by the rough forms, furthermore, appears to be intimately associated with the presence of yet another polysaccharide, the so-called "C" polysaccharide,[19] which is common to the rough forms and which, like Type IV polysaccharide, hydrolyzes to an amino sugar but also contains phosphoric acid. The conversion of pneumococcus types, then, would appear to be an alteration in the ability of the microorganism to synthesize one or another of the type-specific polysaccharides (p. 340). The immunological specificity of other bacteria, including types A, B, and C of Friedländer's bacillus, certain of the meningococcus types, staphylococci, streptococci and other microorganisms, is similarly associated with the presence of polysaccharide haptenes. The nature of the linkage between polysaccharide and antigenic protein in the bacterial cell is not known.

Recent work has shown that an effective antigen need not contain intact polysaccharide; Goebel[20] has prepared an azoprotein antigen from horse serum and the glucoside of cellobiuronic acid whose antiserum will protect mice against Type III pneumococcus infection. Similar protection is afforded against Type II infection by an antiserum to an azoprotein antigen prepared from the glucoside of a *synthetic* isomeric aldobionic acid, gentiobiuronic acid (6-β-glucuronosido-glucose).[21]

Lipoids.[22]—Principally because of the apparent solubility of Forssman antigen in organic solvents and the immunological activity of certain lipoidal preparations from tubercle bacilli it has been thought that lipoids might function either as true antigens (see below) or as haptenes. The experimental evidence, *e. g.*, immunization with mixtures of foreign serum and such substances as lecithin, cephalin and cholesterol, has, however, been inconclusive. It is, therefore, uncertain as yet whether or not lipoids may determine the immunological specificity of antigenic proteins. In this connection it is of some interest that recent work[23] has indicated that the Forssman antigen, generally regarded as the best example of the immunological activity of lipoids, contains polysaccharide haptene. In higher animals the polysaccharide-protein antigen contains lipoidal substances, but these are lacking in heterophile antigen of bacterial origin.

[19] Tillet, Goebel and Avery: Jour. Exp. Med., 1930, *52*:895.

[20] Goebel: Jour. Exp. Med., 1939, *69*:353.

[21] *Ibid*, 1940, *72*:33.

[22] The question of the antigenicity of lipids is reviewed by Weil: Bact. Rev., 1941, *5*:293.

[23] Landsteiner and Levine: Jour. Immunol., 1932, *22*:75. See also Meyer and Morgan Brit. Jour. Exp. Path., 1935, *16*:476.

Non-protein Antigens.—Although in general only proteins and protein-haptene complexes will stimulate the formation of antibodies, there is some evidence that certain non-protein substances may act as true antigens. Such evidence is, however, difficult to interpret in many instances because of the minute amounts of antigenic protein that are required to elicit an immunological response—according to Wells[24] as little as 0.00005 milligram of crystalline egg albumin is effective—which may be present as contamination in naturally occurring non-protein substances. A second possibility that is difficult to eliminate is that of the combination of a non-protein substance with body protein of the animal into which it is injected, leading to the formation of an antigenic complex. It is well known that such simple compounds as formaldehyde will act in this manner, and drug idiosyncrasies are not infrequently accounted for on this basis.

Glucosides.—It was early reported by Ford that the glucoside hemolysin present in the toadstool (*Amanita phalloides*)[25] and the toxin of poison ivy (*Rhus toxicodendron*)[26] also a glucoside, would, upon injection, stimulate the production of antibodies. In recent years Boivin and Mesrobeanu[27] have shown that gluco-lipoids, consisting of a complex polysaccharide linked to a phosphatide or nucleotide component, present in certain of the colon-typhoid-dysentery group of bacteria and apparently representing the endotoxin of these organisms, are antigenic and give rise to antitoxic sera when injected into experimental animals. Evidence such as this suggests that under certain circumstances such non-protein substances may function as true antigens although they appear to be "poor" antigens in that the antibodies produced are generally present only in low titer.

Polysaccharides.—Considerable interest has attached to the possibility that the high molecular weight polysaccharide haptenes may act as antigens apart from the protein molecules to which they lend immunological specificity. Although earlier studies indicated that pure polysaccharides, those of the pneumococcus types, functioned only as haptenes, more recent work has suggested that these substances may be complete antigens and capable of inducing an immunological response. Their immunological behavior is peculiar in some respects,[28] but it appears definitely established that these substances will stimulate the formation of antibodies. The antigenicity of other polysaccharides, such as the vegetable starches, is not established by unequivocal experiment, and for the present these substances may be regarded as not antigenic.

Lipoids.—As indicated above, lipoids apparently do not function as haptenes and, as might be inferred, cannot function as antigens. The antigenic activity of ethereal extracts of tissues containing Forssman antigen and that of similar preparations from the tubercle bacillus and other acid-fast bacteria may be accounted for on the basis of protein impurities. More

[24] Wells: *loc. cit.*

[25] Ford: Jour. Inf. Dis., 1906, *3*:191; *ibid.*, 1907, *4*:541.

[26] Ford: Jour. Pharmacol., 1910, *2*:145.

[27] Boivin and Mesrobeanu: Rev. Immunol., 1935, *1*:553; *ibid.*, 1936, *2*:113; *ibid.*, 1937, *3*:319; Ann. Inst. Pasteur, 1938, *61*:426.

[28] *Cf.* Avery and Goebel: Jour. Exp. Med., 1933, *58*:731; Horsfall and Goodner: Jour. Immunol., 1936, *31*:135.

specifically, the lipid portion of the somatic antigen of the dysentery bacilli does not appear to contribute to its antigenicity or specificity.[29]

The Antigenic Structure of Bacteria.—It is already apparent that the cell substance of a bacterium is not immunologically homogeneous but consists of a number of different antigenic components. The particular combination of antigens, one or more of which may be shared by closely related forms, that goes to make up the immunological character of a bacterial species has been aptly termed an *antigenic mosaic*. This antigenic mosaic is undoubtedly an expression of deep-seated characters that contribute to the biological individuality of the bacterial species, for, from the biological point of view, these antigenic substances are qualitatively different kinds of protoplasm. In this light the immunological method may be regarded as an analytical method, remarkably sensitive, highly specific and crudely quantitative.

In a number of cases these antigens have been separated by biochemical methods and something of their nature is known. The polysaccharide haptenes referred to above are of very considerable immunological importance in many, but not all, cases. The isolation of such substances is relatively simple, usually involving a primary alcohol precipitation from solution of the bacterial cell substance. The somatic antigens of the enteric bacilli, which appear to be identical with endotoxin in many cases, may be prepared by extraction of the intact cells with M/2 trichloracetic acid in the cold,[30] by extraction of the intact cells with glycols such as diethylene glucol,[31] by fractionation of tryptic digests of the cells,[32] or by dissociation and extraction in 6 M urea.[33] These substances may be precipitated from the crude extract by alcohol and further purified. Though it has been indicated earlier that these substances are polysaccharide-lipid complexes, there is not complete agreement on this point. Preparations made by primary trichloracetic acid extraction do appear to be of this nature but those isolated by glycol extraction are polysaccharide-lipid-polypeptide complexes. These may be dissociated and reconstituted in hot formamide solution and the polypeptide portion seems to be essential to antigenicity. Sharp separation of the immunological specificity of the flagellar antigens has not been possible by chemical means, but physical separation in which the flagella are broken off by shaking and separated from the cell proper by differential centrifugation has been accomplished. So far as is known, the flagellar antigens are protein in nature. In the past a good deal of work has been done with "nucleoprotein" separated from the bacterial cells by extraction with dilute alkali. The immunological entities have not been separated in such preparations, however.

The number of antigens that can enter into this mosaic in a single species is unknown; some *Salmonella* species have been found to contain seven or eight separate antigens which differ from one another not only immunolog-

[29] Partridge and Morgan: Brit. Jour. Exp. Path., 1940, *21*:180.
[30] Boivin and Mesrobeanu: Rev. Immunol., 1935, *1*:553.
[31] Morgan: Biochem. Jour., 1937, *31*:2003.
[32] Raistrick and Topley: Brit. Jour. Exp. Path., 1934, *15*:113.
[33] Walker: Biochem. Jour., 1940, *34*:325.

ically but also in their resistance to heat, alcohol and the like, and these and other bacteria will undoubtedly prove to be increasingly complex with further study. The number of antigens demonstrable by antigenic analysis (p. 269) is, of course, only a minimum since a given antigen remains an entity only so long as possible components of it have not been found to occur separately. It is of some interest that antigens co-existing in the same cell may not be equally demonstrable. The species specificity observable in rough pneumococci, for example, is present in the smooth forms but is masked by the predominant type specific antigens. Similar phenomena have been observed in a variety of bacterial species. Conversion to varying degrees of roughness may bring to light "new" antigens which, though presumably constantly present, have been masked by the presence of other antigenic substances. There is a tendency to interpret such findings as indicating the relative position of antigenic substances within the cell, the peeling off of "outer" antigenic layers and exposure of secondary and tertiary layers of antigen within the cell. While there is something to be said for this concept as a figurative mode of expression, it may be pointed out that, aside from the fact that in some bacteria specific antigen is present in the capsule or flagella, there is no evidence of an immunological geography in the bacterial cell.

The demonstration of the occurrence of common antigens in heterologous bacteria may be interpreted in two ways. Thus it may be assumed that the identical antigen, or at least its determinant portion, is shared. This is a common view and one for which there is a good deal of justification in many instances. It is believed by some, however, that the observed cross reactions are attributable to the occurrence of similar determinant groups and the type of partial antigen-antibody reaction observed with artificial antigens. As yet there is no definitive evidence to substantiate either view.

ANTIBODIES

The immunological response of an animal to the initial injection of an antigenic substance is not immediate but after a suitable time interval or incubation period is manifested as an alteration in the properties of the blood serum with respect to the antigen. An immune serum, or antiserum, differs from normal serum in that it reacts, either *in vivo* or *in vitro*, with the homologous antigen. This property of immune serum is a consequence of the presence of *antibodies*, substances which are formed by the animal body in response to the presence of antigen in the tissues, and which combine specifically with the antigen. The antigen-antibody reaction is demonstrable in a number of ways, the particular technique employed depending upon the nature of the antigen. By such means five apparently different kinds of antibodies may be found, although, as will appear, these are probably but a single substance. They are:

(1) the *antitoxins*—antibodies formed in response to the injection of toxins which, when mixed with the homologous toxin, neutralize its poisonous qualities;

(2) the *lysins*—antibodies which bring about a dissolution or lysis of bacterial and other cells;

(3) the *opsonins*—antibodies which sensitize bacterial cells in such a way that they are readily engulfed by the phagocytic cells;
(4) the *agglutinins*—antibodies formed in response to the injection of bacterial cell substance which, when mixed with the homologous microorganism, immobilize the bacteria if they are motile, then aggregate, or agglutinate, the cells with the formation of clumps which settle out of suspension;
(5) the *precipitins*—antibodies formed in response to the injection of soluble antigens such as proteins which, when mixed with soluble antigen, aggregate the molecules with the formation of a precipitate.

In addition to these five generally recognized kinds of antibodies, two others may be provisionally added:

(6) *ablastins*—reproduction-inhibiting antibodies which prevent the multiplication (cell division) of the invading microorganism;
(7) *neutralizing antibodies*—antibodies which, when mixed and incubated with the infectious agent, generally a filterable virus, render it non-infective.

The various antibodies may be considered briefly one by one.

Antitoxins.—It was found by von Behring and Kitasato[34] in 1890 that the immunity of rabbits and mice which had been immunized against tetanus was associated with the ability of the blood serum to neutralize the toxic substances produced by the tetanus bacillus. The substance in the serum which neutralized the tetanus toxin was designated by these workers as *antitoxin.* Subsequent investigation has shown that the animal body forms antitoxins in response to the injection of a variety of antigenic poisons, not only those of bacterial origin such as diphtheria toxin, botulinus toxin and the like, but also against the phytotoxins and zootoxins (p. 176).

The action of antitoxin may be directly demonstrated in the following way: if a fatal, or many times fatal, dose of toxin be mixed with an appropriate amount of antitoxic serum *in vitro*, the injection of the mixture into a susceptible animal is wholly without injurious effect; the poisonous qualities of the toxin are nullified by the immune serum. The reaction is, like all other immunological reactions, highly specific, and an antitoxin which neutralizes the homologous toxin is without effect on heterologous toxins. The nature of the effect of the antitoxin which renders a powerful toxin pharmacologically inert is unknown.

The Toxin-Antitoxin Reaction.—The combination of toxin and antitoxin does not necessitate the complete destruction of either component; neutral mixtures of toxin and antitoxin may be dissociated by treatment with hydrochloric acid, by freezing in the presence of phenol or tricresol and, to some extent, by simple dilution. In certain cases, *e.g.*, pyocyaneus toxin and certain snake venoms, in which the toxin is more resistant to heat than the antitoxin, the latter may be selectively destroyed and the neutral mixture becomes toxic upon judicious heating. It appears, therefore, that a more or less loose combination of toxin and antitoxin takes place, the poisonous properties of the toxin being held in abeyance as long as the union persists.

[34] von Behring and Kitasato: Deut. med. Wchnschr., 1890, *16*:1113.

The rate of reaction between toxin and antitoxin, like the chemical reactions, is dependent upon temperature, concentration, character of the medium in which the reaction occurs, and similar factors. The avidity of an antitoxin for its corresponding toxin differs in different cases; the union between tetanus toxin and antitoxin, for example, takes place less rapidly than that between diphtheria toxin and antitoxin.

An understanding of the precise character of the toxin-antitoxin reaction is dependent upon the interpretation of phenomena revealed by quantitative studies. It might be expected that a given quantity of antitoxin would always neutralize a constant amount of toxin, that the neutralization would follow the law of multiple proportions. This is, however, not the case, and it appears that the amount of antitoxin required to neutralize a given quantity of toxin is dependent upon (*a*) the manner in which the two are mixed with one another and (*b*) the relation between toxicity and combining power in the particular filtrate under consideration.

In the first instance it has been observed that when an excess of toxin is added to its specific antitoxin in several portions at proper intervals of time, much more unneutralized toxin remains in the mixture than if the same quantity of toxin had been added to the same quantity of antitoxin at one time. This is known as the *Danysz phenomenon.* If, on the other hand, antitoxin be added to toxin in successive equal portions, it may be shown that, in general, the first portion of antitoxin neutralizes a greater portion of the toxin than the second, the second a greater than the third, etc. In the second case it has been found that there is no constant relationship between toxicity and combining power of a toxic filtrate; toxicity slowly diminishes upon storage but combining power remains unchanged. A similar change may be brought about by various physical and chemical agents, notably formaldehyde, which destroys toxicity but does not affect the antitoxin combining power of the preparation. Such altered toxin, which retains its antigenicity unchanged in addition to its combining powers, is designated as *toxoid* and, when prepared by treatment with formaldehyde, *formol toxoid* or *anatoxin.* The proportions of toxin and toxoid in a given filtrate are variable. Consequently there is no fixed relation between toxicity and power to combine with antitoxin; the MLD's neutralized by a unit quantity of antitoxin may vary from one filtrate to another or in the same filtrate at different times from 30 to 130.

Three theoretical explanations of the toxin-antitoxin reaction have been advanced. They are known by the names of their proponents, Ehrlich, Arrhenius and Madsen, and Bordet and Landsteiner.

According to Ehrlich the reaction is purely chemical and essentially similar to the neutralization of a strong acid by a strong base. The combining properties of toxin and antitoxin would, therefore, be a manifestation of primary valencies. This concept is, however, not compatible with the observed behavior of toxin and antitoxin in mixture; the Danysz phenomenon, for instance, suggests that if the neutralization proceeds according to Ehrlich, the reacting substances are not homogeneous but consist of mixtures of substances with varying affinities for one another. On the basis of his studies on diphtheria toxin, Ehrlich postulated a series of components

of toxin which differed from one another both in their avidity for antitoxin and in their toxicity.

Arrhenius and Madsen preferred to regard the toxin-antitoxin combination as essentially a chemical reaction but analogous to the neutralization of a weak acid by a weak base (ammonia and boric acid). Although this hypothesis is attractive in many respects, it is not compatible with certain aspects of the toxin-antitoxin reaction. Simple dilution of a neutral mixture, for example, does not result in the degree of dissociation that this theory calls for. As a consequence of this and certain other discrepancies, the theory of Arrhenius and Madsen is, like that of Ehrlich, not generally accepted at the present time.

A third concept, proposed by Bordet and strongly supported by Landsteiner, is that of the toxin-antitoxin reaction as an adsorption phenomenon, essentially physico-chemical in nature and arising as a consequence of intermolecular forces (secondary valencies). The varying degrees of toxicity apparent in the neutralization of toxin may be regarded as due to differences in completion of saturation of the individual toxic units, a process that may be compared to certain staining reactions such as the action of iodine upon starch, a dilute iodine solution producing a light blue tinge, a stronger solution a deep blue. This adsorption theory would view the action of antitoxin upon toxin as a sort of progressive attenuation, proportional to the amount of antitoxin added. The evidence for such a mechanism, not only in the toxin-antitoxin reaction but in antigen-antibody reactions in general, is very strong indeed, and it is generally regarded as highly probable that these reactions are essentially physico-chemical in nature. This view of the immunological reactions will be considered at greater length in a later section.

The Standardization of Antitoxins.—The quantitative evaluation of the toxin-neutralizing capacity of antitoxic sera is clearly a matter of considerable practical as well as theoretical importance. Ehrlich originally proposed as the standard unit of diphtheria antitoxin that amount which would just neutralize 100 guinea pig MLD's of toxin. As indicated above, however, variability in the relation of toxicity to combining power invalidates any standard based on toxicity; in other words, the combining power of a toxin is not a measure of its toxic qualities. On the other hand, since the combining power of a toxic filtrate remains constant within narrow limits, it is possible to establish an arbitrary standard unit upon which the relative strength of all antitoxic sera can be based. Such a standard diphtheria antitoxin was first prepared by Ehrlich and was preserved by him with all precautions against possible deterioration. A standard antitoxic serum based on Ehrlich's arbitrary standard unit is also prepared in this country by the National Institute of Health of the United States Public Health Service, and is distributed every two months to the licensed producers of commercial serum. The unit is international, standard sera being tested from time to time by the Biological Standardization Commission of the League of Nations.[35]

Three methods are used in the titration of diphtheria toxin and antitoxin.

[35] *Cf.* Amer. Jour. Public Health, 1935, *25*:712.

The first of these is the classic method of Ehrlich in which two limits (Lat., *limes*) are determined by guinea pig inoculation. These are the L_o dose of toxin, which is defined as that amount exactly neutralized by the standard unit of antitoxin, and the L+ dose, that amount of toxin which, when mixed with one unit of standard antitoxin is just sufficient to kill in four days a guinea pig approximately 250 grams in weight. With these limits established, the serum to be standardized is mixed with the toxin just titrated, and the smallest amount of serum which, when mixed with the L+ dose of toxin, will produce a 50 per cent mortality in the inoculated guinea pigs is considered to contain one unit of diphtheria antitoxin.

A second method is based upon the observation that the intradermal injection of 1/250 to 1/500 MLD of diphtheria toxin into a guinea pig is followed by a local reaction, swelling and erythema and, with slightly larger amounts of toxin, necrosis, a phenomenon sometimes called the *Römer reaction.* By the use of such intradermal inoculations an Lr dose of toxin may be determined, *i.e.*, that amount of toxin which, when mixed with one standard unit of antitoxin, will produce the minimal skin reaction. The serum to be standardized is mixed in varying amounts with Lr doses of toxin, and that amount of serum which gives the minimal skin reaction is considered to contain one unit of antitoxin.[36] This method has the advantage of allowing the testing of a number of toxin-antitoxin mixtures in one animal but has not displaced the classic method in common usage.

The third method makes use of the *Ramon flocculation*, the *in vitro* precipitation of toxin and antitoxin when mixed in optimal proportions.[37] Standard antitoxin is mixed with varying quantities of toxin, and the tube first showing precipitation contains one Lf dose of toxin. Varying amounts of the serum to be standardized are mixed with the Lf dose of toxin. The amount of serum in the tube first showing flocculation in this second series is considered to contain one unit of antitoxin. This method differs from the other two in that it depends upon the combining power of a toxic filtrate rather than on toxicity. It is generally used, not as a final method of standardization, but as a preliminary to standardization by the Ehrlich method.

The interrelationships of these limits are of some interest. The L+ dose is, of course, larger than the L_o dose, and, because of the peculiarities of the toxin-antitoxin reaction, by considerably more than one MLD. The Lr dose approximates the L_o dose as might be expected in view of the small amount of toxin required to elicit the skin reaction. The Lf dose is generally somewhat less than any of these, since it is a measure of combining power rather than toxicity and is unaffected by differences in the proportions of toxin and toxoid. It would appear that the Lf/Lr ratio should be the same for a given toxic filtrate immediately upon standardization. It has been found, however, that this ratio differs with different antitoxic sera. This appears to be a consequence of differences in avidity, not of hypothetical

[36] Glenny and Allen: Jour. Path. and Bact., 1921, *24:*61.
[37] Ramon: Compt. Rend. Soc. Biol., 1922, *86:*661, 711, 813.

toxin components as suggested by Ehrlich, but of antibody. The avidity of antitoxic sera is associated with the protein fraction containing the antibody; successive globulin fractions differ from one another in avidity[38] (see also p. 283).

The Bactericidal Substances—Lysins.—It was early shown by Nuttal[39] that fresh, defibrinated animal blood is markedly bactericidal. This property, also possessed by cell-free serum, was found to be heat labile and destroyed by holding at a temperature of 55 to 56° C. for thirty minutes. Such heated sera are said to be *inactivated.* The blood or serum from a single animal species is not equally active on all species of bacteria and, conversely, a given bacterial species is affected to varying degrees by the blood of different animal species. It was suggested by Buchner[40] that the natural resistance of an animal to infection could be explained in part by this bactericidal quality of the blood, and he proposed the name *alexin* (Gr., to ward off) for the heat labile activity.

Although this bactericidal activity of the blood and serum of normal animals is frequently specifically increased by immunization, such an increase does not invariably accompany the development of the immune state. The serum of guinea pigs immunized with *Vibrio metchnikovii* is strongly germicidal for that microorganism, while that of unimmunized guinea pigs is devoid of specific bactericidal quality. On the other hand, immunization with streptococci, while producing a specific resistance to infection with the bacterium, frequently fails to induce an increased, specific germicidal activity.

The Pfeiffer Phenomenon.—The bactericidal effect is, in some instances, accompanied by visible dissolution of the bacterial cells. The course of events following the introduction of cholera vibrios into the peritoneal cavity of an immunized animal was followed microscopically by Pfeiffer[41] and described by him in detail. The vibrios first lose their motility, then swell up and crumble into small fragments. The dissolution of the fragments follows, and no trace of the bacterial cell remains visible. This action of the body fluids is attributable to the presence of an antibody termed a *lysin* (Gr., to loose, dissolve). It occurs not only within the peritoneal cavity of an immunized animal, but also when the peritoneal fluid or blood serum is removed from the body and brought immediately in contact with the bacteria *in vitro.* The lytic activity of bactericidal sera *in vitro* is heat labile and the process of lysis appears to be essentially identical with that observed *in vivo.*

Visible lysis of bacterial cells, however, does not invariably accompany the lethal activity of a bactericidal serum. In fact, the majority of bacteria are not dissolved by immune sera in the manner described above. This is not to be taken to indicate that the processes are fundamentally different; not only are many bacteria highly resistant to visible structural alterations,

[38] *Cf.* Barr and Glenny: Jour. Path. and Bact., 1931, *34*:539; Brit. Jour. Exp. Path., 1931, *12*:337; Glenny *et al.:* Jour. Path. and Bact., 1932, *35*:495. Kekwick, Knight, Macfarlane and Record: Lancet, 1941, *1*:571.

[39] Nuttall: Ztschr. f. Hyg., 1888, *4*:353.

[40] Buchner: Arch. f. Hyg., 1891, *10*:84.

[41] Pfeiffer and Issaeff: Ztschr. f. Hyg., 1894, *17*:355.

viz., the lack of sensitivity to wide variations in osmotic pressure, mechanical pressure, etc., but, as will appear, the antigen-antibody reaction takes place in two stages, the first the union of antigen and antibody and the second the visible consequences of that union. Whether or not dissolution of the bacterial cells occurs, it may be readily shown that they unite with the lysins.

Hemolysis.—The lysis of bacteria by an immune serum is not a unique reaction in which only bacterial cells may play a part; bacteriolysis is, rather, a special case of a general phenomenon, for immunization with a variety of cells results in the production of cytolytic sera. Of these, erythrocytes have been by far the most widely studied, for the lysis of these cells, hemolysis, is readily apparent in the test tube; the red opacity of the cell suspension changes to the clear red solution of hemoglobin as lysis proceeds. The stroma is not dissolved but, upon examination, appears misshapen. First, demonstrated by Bordet,[42] hemolysis has been particularly useful not only as a type of lytic reaction peculiarly suitable for laboratory manipulation, but also as an indicator of antigen-antibody combination when visible lysis does not or cannot take place. It may be noted that the immune hemolysins, *i.e.*, those formed by the animal body in response to the injection of erythrocytes, are to be distinguished from the filterable hemolysins formed by bacteria (p. 177).

Antisera have been prepared against a variety of other cells. The injection of spermatozoa leads to the appearance, in the serum of the inoculated animal, of a spermatoxic substance that first renders the corresponding spermatozoa motionless and then kills them. A number of similar cytolytic sera have been prepared—"nephrotoxic" sera by the injection of kidney cells, "hepatotoxic" sera by the injection of liver cells, etc., but the organ specificity claimed for these last has not been satisfactorily demonstrated.

The Mechanism of the Lytic Reaction.—An observation contributing in large measure to a partial understanding of the mechanism of the action of the immune lysin was that lytic or bactericidal activity could be completely restored to an inactivated serum by the addition of a small amount of fresh unheated serum, either normal or immune. It appears, therefore, that the lytic reaction is a consequence of the interaction of three components, rather than two as in the case of the toxin-antitoxin reaction. One of these is clearly an immune body, *i.e.*, is formed in response to the injection of the antigen, and another, the heat labile component present in normal as well as immune serum. The third component is, of course, the antigen.

The heat labile component was termed *alexin* by Buchner,[43] as indicated above, and renamed *complement* by Ehrlich. The immune body or antibody was called *substance sensibilisatrice* or *sensitizer* by Bordet and *amboceptor* by Ehrlich. The relation between these components may be expressed in tabular form for a hemolytic system:

[42] Bordet: Ann. Inst. Pasteur, 1898, *12*:688.

[43] Buchner originally used the term alexin to designate the entire bactericidal action of the blood serum; alexin was later used by Bordet for the heat labile substance alone; and at the present time alexin is regarded as synonymous with complement.

erythrocytes (antigen)	+ unheated immune serum (complement or alexin + amboceptor or sensitizer)			= hemolysis
erythrocytes (antigen)	+ heated immune serum (amboceptor or sensitizer)			= no hemolysis
erythrocytes (antigen)	+ heated immune serum (amboceptor or sensitizer)	+	unheated normal serum (complement or alexin)	= hemolysis

These components react with one another, not at random but in an orderly manner; the union between antigen and amboceptor must precede the reaction with complement. Complement does not combine with antigen in the absence of amboceptor, but antigen and amboceptor will unite regardless of the presence of complement. This may be demonstrated directly by using mixtures of only two components or in the presence of all three by holding the mixture at 0° C.; the antigen-amboceptor union readily takes place at this temperature, but complement unites only slowly and may be demonstrated in the supernatant following centrifugation.

According to Ehrlich, complement acts upon the antigen only indirectly through the amboceptor, the last functioning as a bridge between the first two. In this connection it is of interest that colloidal silicic acid may act as a sensitizing agent in the lysis of red cells. The evidence does not require that the action of complement be indirect in this way; it is established only that the antigen must be sensitized by the antibody before union with complement can occur. According to Bordet the union is in the nature of a specific adsorption, the sensitized antigen being rendered susceptible to the lytic action of the complement. The evidence, which cannot be considered at length here, strongly supports the latter view. In consequence it is generally admitted that the terms alexin and sensitizer are preferable to complement and amboceptor, although the latter remain in common usage. Quantitative studies by Heidelberger and his co-workers[44] have shown that under ordinary circumstances about seven molecules of complement are required for each molecule of antibody, but in very dilute solution the ratio may approach unity. These workers believe that complement exists in a loose union with antibody but is tightly bound in the antigen-antibody complex.

The Neisser-Wechsberg Phenomenon (Complement Deviation).—It has been observed by Neisser and Wechsberg[45] that when varying amounts of immune serum (amboceptor) are added to constant amounts of normal serum (complement) and antigen, there is, as might be expected, no lysis with very small amounts of amboceptor, but as larger amounts are added, lysis takes place. When, however, amboceptor is added in considerable excess, lysis again fails to take place. From these and similar experiments it was concluded that a "deviation of complement" occurs under conditions where the amboceptor is in great excess, *i.e.*, the complement unites with

[44] Heidelberger: Jour. Exp. Med., 1941, *73*:681; Heidelberger, Weil and Treffers: *ibid.*, 1941, *73*:695; Heidelberger, Rocha e Silva and Mayer: *ibid.*, 1941, *74*:359.

[45] Neisser and Wechsberg: Münch. med. Wchnschr., 1901, *48*:697.

the unbound amboceptor rather than with the amboceptor which has united with the antigen. A special explanation need not be devised for this phenomenon, however, for a similar failure of antigen-antibody union to occur is apparent in other serological reactions in the region of antigen or antibody excess. Such, for example, is the prozone phenomenon (p. 269) in the precipitin and agglutination tests. The same effect may be observed *in vivo*, for successful protection of mice against pneumococcus infection has long been known to be dependent upon optimal amounts of antiserum and culture; otherwise a zonal phenomenon in which there is no protection (the *Schwellenwert* of Neufeld and Haendel) may be encountered.

The Bordet-Gengou Phenomenon or Complement Fixation.—As pointed out above, the lytic action of an immune serum is difficult to observe with many bacteria and cannot take place with an antigen such as egg albumin. Whether or not the phenomenon of lysis occurs or is made evident through some observable change in the antigen, it is possible to demonstrate the union of antigen and antibody by the addition of an indicator system. The lysis of red blood cells is used for this purpose and is commonly referred to in this connection as the hemolytic system; sheep cells and sheep hemolysin are generally used.

The antigen and inactivated serum (either of which may be unknown) are mixed together with the proper amount of complement in the form of fresh, unheated guinea pig serum. If the antigen and the inactivated serum unite, the complement will combine with the sensitized antigen and is said to be "fixed." When the hemolytic system is added in the form of heated hemolytic serum and erythrocytes, no complement is available, hemolysis does not occur, and the test is positive. If, however, the antigen and inactivated serum do not react, the complement remains free and combines with the sensitized red cells, hemolysis results and the test is negative. This phenomenon may be illustrated in the following way:

antigen	positive		negative	sheep red cells
+	←———	complement	———→	+
inactivated serum				inactivated hemolytic serum (amboceptor)

The quantitative aspects of the test are obviously of primary importance, and each reagent must be titrated and added in the proper amount.

The complement fixation test may be used with a known antiserum for the identification of an unknown bacterium or with a known antigen as a means to detect antibodies in an unknown serum. Perhaps the commonest application of the test is in the diagnosis of syphilis, where it is known as the Wassermann test.

The Nature of Complement.—It is likely that the process of lysis is accomplished by complement, the antibody simply sensitizing the antigen to this lytic action. Complement is not increased during immunization, and a high titer immune serum may be only feebly lytic because there is not sufficient complement present to utilize the excess of amboceptor. It is of some interest that there is little species specificity in complement; that from guinea pig serum, for example, may bring about the lysis of beef cor-

puscles in the presence of amboceptor in goat serum. Some quantitative differences are apparent, however, for a serum which may be actively complementary in some reactions may be relatively inactive in other combinations.

The property of inactivation by heat has been referred to above. Complement is also inactivated by shaking, but in neither case is the inactivation completely irreversible, for some activity may be regained on standing. In this respect complement behaves as a typical hydrophilic colloid which can be made to aggregate by physical forces but shows a tendency to spontaneous dispersion and restoration to the original state. The activity disappears upon standing—rapidly at room temperature (two or three hours) and more slowly in the ice-box, where it may be preserved for three or four days. Complement is irreversibly destroyed by strong acids or alkalis and reversibly inactivated by ions such as Mg, Ca, Ba, Sr and SO_4 or by hypertonic salt solutions. In this connection it is of some interest that complement which is inactivated by raising the salt concentration to 5 to 10 per cent may be preserved at low temperatures in this form for several weeks, the activity being regained upon dilution with distilled water. These properties and some others, such as ready adsorption on surfaces, suggest a close relationship between complement and the enzymes; it has also been pointed out that there are remarkable resemblances to certain compounds of protein with soaps and lipoids. There is a close association of complement with the blood-clotting mechanism and some workers believe it to be identical with prothrombin. The nature of the lytic action of complement is as yet, however, purely speculative.

Complement is intimately associated with the serum proteins and there is reason to believe, *viz.* the destruction of complement by trypsin and the antigenicity of the activity, that this lytic agent is protein in nature. Complement may be split into two parts by the separation of serum protein into albumin and globulin. The globulin fraction will unite with sensitized cells although no lysis occurs, and is called the *mid-piece,* while the albumin fraction which will not unite with the sensitized antigen in the absence of the mid-piece but produces lysis when it unites with the sensitized cell-mid-piece complex is termed the *end-piece.* Other investigations have indicated that complement may be split up into four components by other fractionation methods. These are:[46]

C'_1 or mid-piece which is precipitated from guinea pig serum by passing CO_2 through the serum diluted 1:10 with distilled water, or by dialysis against distilled water. This component is destroyed at 56° C in 30 minutes.

C'_2 or end-piece which remains in solution after the C'_1 fraction is precipitated. It shows the same heat lability.

C'_3 is inactivated by yeast, zymin or cobra venom and is not destroyed at 56° C in thirty minutes.

C'_4 or fourth component is specifically inactivated by treating guinea pig serum with dilute ammonia, hydrazine or viper venom, or by shaking with chloroform or ether. It is also heat stable but this stability is greatly reduced in the presence of 10 per cent sodium chloride.

[46] For a discussion of the terminology see Pillmer and Ecker: Science, 1941, *94*:437.

Human complement likewise consists of these four components but all the components are not interchangeable with those of guinea pig complement.[47] According to Pillmer, Seifter and Ecker[48] not all of these components are fixed equally in complement fixation.

Opsonins.—If a mixture of polymorphonuclear leucocytes and bacteria or other particulate matter is incubated for a time, it will be found on microscopic examination that a number of the leucocytes have ingested the foreign particles. Few if any particles will be ingested if the mixture is prepared in physiological salt solution, a considerable number if the fluid is normal serum. In the case of bacteria, great numbers of the microorganisms will be found packed into the leucocytes when the two are suspended in the specific immune serum. The antibodies present in the immune serum which so remarkably stimulate this engulfment by body cells are designated *bacteriotropins*, a term not in common use, or *opsonins*. The term opsonin was originally used to designate the activity of normal serum, hence the antibodies in the immune animal are sometimes called immune opsonins. The cells which ingest such particulate matter are termed *phagocytes* (devouring cells). This property is not confined to the polymorphonuclear leucocytes or heterophils, although these have been most widely used in *in vitro* experiments because of their availability, but is also present in various mononuclear phagocytes. These cells and their rôle in immunity are discussed in the following chapter.

The Opsonic Index.—A quantitative estimate of opsonin present in a given immune serum may be made by comparing the number of bacteria ingested by normal leucocytes in normal serum with the number ingested by normal leucocytes in the immune serum. The appropriate mixtures are prepared and incubated in capillary tubes, smears made and stained, and the bacteria engulfed by an arbitrary number (usually 50 or 100) of leucocytes counted.[49] The average number of bacteria per leucocyte, or *phagocytic index*, is determined for the normal and immune sera, and the ratio of the phagocytic index of the immune serum to that of the normal serum is termed the *opsonic index*.

The opsonins may be estimated also by the dilution method; specimens are prepared in the usual way, except that the normal and patient's sera are diluted with saline or Ringer's solution. One mixture is made with salt solution or Ringer's solution to determine the degree of spontaneous phagocytosis. The dilution of serum which gives the same amount of phagocytosis as a mixture without serum is taken as the end-point.

The Factors Influencing Phagocytosis.—The process of phagocytosis is markedly influenced by environmental factors and the nature of the bacterium and leucocytes used, as well as by the amount of opsonin present. Departures from a neutral or very slightly acid reaction or from isotonicity, and the presence of certain ions, notably the citrate radicle, depress the

[47] Ecker, Pillmer and Seifter: Jour. Immunol., 1943, *47*:181.

[48] Pillmer, Seifter and Ecker: Jour. Exp. Med., 1942, *75*:421.

[49] The details of this technique may be found in Wright and Colebrook: *Technique of the Teat and Capillary Glass Tube*. Constable and Co., Ltd. London. 2nd ed. 1921; Fleming: *A System of Bacteriology*. Medical Research Council, London, 1931, *9*:212.

degree of phagocytosis. The last is of practical significance in that it contraindicates the use of citrated blood. The presence of calcium, on the other hand, may restore phagocytic power to leucocytes which have been allowed to stand in isotonic salt solution for a number of hours, a point of some interest in connection with the apparent intimate relation between this ion and cell division and the ability of ameboid cells to form new surfaces.

The nature of the bacterium to be ingested is of considerable importance; in general, virulent forms are relatively resistant to phagocytosis. It is not unlikely that this resistance is associated with the presence of a capsule; in the case of the pneumococcus, for example, not only are the smooth, encapsulated forms highly resistant to phagocytosis, but the presence of

Fig. 29.—Phagocytosis of pneumococci by culture macrophages. *1*, Phagocytosis in presence of normal serum; relatively few bacteria have been ingested. *2* and *3*, Phagocytosis in the presence of antipneumococcus serum; enormous numbers of pneumococci have been ingested and appear as agglutinated masses. The lightly staining forms are degenerating. Hematoxylin and eosin-azure II. $\times$ 1200 (Zuckerman).

capsular polysaccharide markedly inhibits phagocytosis by an immune serum, presumably through combination with the antibody. Bacteriophage (p. 863) renders bacteria considerably more sensitive to phagocytosis.

It has been shown by a number of investigators, especially Hektoen[50] and his associates, that the phagocytic power or activity of the leucocytes is subject to considerable variation independent of variation in the opsonic content of the blood. This inherent phagocytic power of the leucocytes varies, with respect to certain bacteria at least, even in persons apparently in perfect health. In the child at birth the leucocytes are somewhat less active phagocytically than in the adult; they grow less active for a few months, and then more active, the adult standard for streptococci, pneumococci and staphylococci being reached about the third year. In pneu-

[50] Hektoen: Jour. Amer. Med. Assn., 1911, *57*:1579.

monia, scarlet fever and other conditions in which there is acute leucocytosis when the outlook is favorable, the phagocytic power of leucocytes has been found to be greater than normal for the specific bacteria. The increase in activity in such cases may be due to the predominance of young leucocytes.

The Process of Phagocytosis.—The mechanism of ingestion is essentially one of the interplay of interfacial forces. A formulation of the free surface energy relationships at the points of contact between particle and cell and their respective liquid interfaces has been worked out by Fenn[51] and subjected to experimental tests by Mudd[52] and his associates. Low interfacial tension of the bacteria against the leucocytes—and high interfacial tension against the medium—favors ingestion. The immune opsonins, and to some extent the "normal" opsonins, apparently form a surface deposit on the bacterial cells which promotes phagocytosis by altering the interfacial tension in this manner. In addition to surface tension, other factors such as the viscosity of the phagocytic cell substance enter into this phenomenon. Physical tensions are not the sole controlling factors, however, for increased oxygen consumption accompanies the process of ingestion, the rise beginning at once, reaching a maximum value twice that at the start in about fifteen minutes and persisting for 90 to 150 minutes.[53]

It is of some interest that bacterial cells may be artificially "opsonized," *i.e.*, made more readily phagocytable, by treatment with iron ammonium alum, chrome alum, protamine sulfate or gallotannic acid. The effect is reversed by treatment with oxalate but reversion is very difficult when the cells are sensitized with immune opsonin.[54] The significance of such observations in immune opsonization and phagocytosis is not clear.

The Fate of Ingested Bacteria.—Following phagocytosis many, but not all, species of bacteria may be observed to undergo a process of dissolution, with swelling, granulation and fragmentation appearing as successive stages in their destruction. Although it was early supposed that intracellular digestion was no more than intracellular lysis through the agency of the immune lysin and complement which would have occurred whether or not phagocytosis took place, it now seems probable that the two processes are essentially different.

Bacteria are no exception to the rule that living organisms are not subject to attack by digestive enzymes, and the question arises as to whether death is a necessary preliminary to ingestion or whether it may occur within the phagocyte. It is probable that the viability of the microorganism is not an important factor in phagocytosis; in most cases the cell may be engulfed whether it is living or dead. In the case of those bacteria which are destroyed within the leucocyte, killing is necessary before digestion can take place. Killing of a viable organism is accomplished through the agency of endocellular bactericidal substances which have been designated *leukins*. These

[51] Fenn: *The Newer Knowledge of Bacteriology and Immunology*. Jordan and Falk. University of Chicago Press. Chicago. 1928. p. 861.

[52] The process of phagocytosis is discussed at length by Mudd, McCutcheon and Lucke: Physiol. Rev., 1934, *14*:210.

[53] Baldridge and Gerard: Amer. Jour. Physiol., 1933, *103*:235.

[54] Gordon and Atkin: Brit. Jour. Exp. Path., 1938, *19*:204.

substances are not equally active on all species of bacteria, some micro-organisms remaining viable within the phagocyte over considerable periods of time. The ingested bacteria are protected against the action of immune serum, *i.e.*, bactericidal or lytic antibodies, and this is possibly of some significance in the dissemination of bacteria within the host in the event that they remain viable and are later freed from the leucocyte.[55] Leucocytes have been shown to contain a variety of digestive enzymes, including proteases, lipases and various carbohydrate-splitting ferments which are presumably responsible for the actual dissolution of the bacterial cell.

The Nature of the Opsonic Activity.—The opsonins are true antibodies in that they are increased by immunization and exhibit the specificity characteristic of the immune antibodies. The activity is, like that of the lytic sera, made up of two components, one thermostable and the other thermolabile. The thermolabile component is present in normal serum and the

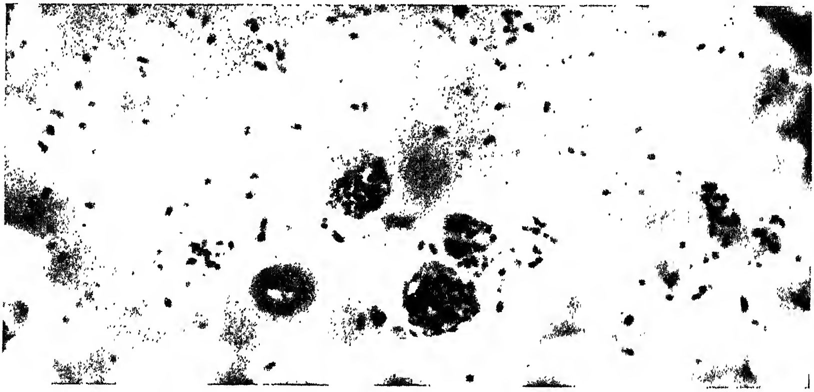


Fig. 30.—The phagocytosis of typhoid bacilli by leucocytes in whole blood. Note the enormous numbers ingested by the white cells and the bacilli lying free. Hasting's stain; × 1200.

reduced opsonic powers of an inactivated immune serum may be restored by the addition of a small amount of normal serum. In this and other respects the thermolabile component of opsonin strikingly resembles complement and in the past has been assumed by many to be identical with this component of the lytic system. It has never been possible, however, to establish this identity by unequivocal experiment and there is some evidence suggesting that the two substances are not identical. For example, the fourth component of complement has been reported[56] to be necessary to the lytic reaction but not required for opsonization. It appears, then, that complement and the thermolabile element of opsonin, while resembling each other closely in many respects, may not be regarded as identical.[57]

[55] Rous and Jones: Jour. Exp. Med., 1916, *23*:601.
[56] Gordon, Whitehead and Wormall: Jour. Path. and Bact., 1929, *32*:57.
[57] See also Ecker, Pillmer and Kuehn: Jour. Immunol., 1942, *43*:245.

There is some question as to whether the thermolabile factor is essential to phagocytosis, for experimental evidence has been presented[58] which indicates that while the thermolabile factor contained in normal serum markedly accelerates the *rate* of phagocytosis, approximately the same number of bacteria are ingested at the end of eight hours by phagocytes in the presence of heated serum alone.

Agglutinins.—If the blood or serum of an animal previously immunized against a bacterium be mixed with a suspension of the microorganisms, the latter become immobilized and in a short time aggregate to form large clumps of cells. In the test tube these clumps settle out, and the turbid bacterial suspension is cleared with the formation of a precipitate-like mass of clumped cells in the bottom of the tube. This phenomenon is termed *agglutination* and the bacterial cells are said to be *agglutinated.* The bacteria are not killed by agglutination and will, in fact, grow in immune serum although with altered morphology and the formation of long chains of bacillary forms—the so-called "thread reaction" of Pfaundler. Living bacteria need not be used for the agglutination reaction, for dead bacteria are agglutinated as readily as the viable forms.

Although many species of bacteria may be clumped by "normal" sera in low (1 : 5 to 1 : 10) dilutions, the capacity of a serum to agglutinate bacteria is greatly enhanced by immunization; high-titered antisera may be prepared which will bring about agglutination in dilutions of 1: 20,000 to 1 : 50,000. The agglutination reaction is, then, an antigen-antibody reaction and the antibody is designated an *agglutinin.* The *antigen* is sometimes termed an *agglutinogen.* The agglutinin does not require the cooperation of complement or other heat-labile substances and inactivated sera will agglutinate to titer.

Not only bacteria but a variety of free cells, including erythrocytes and others, are agglutinated by normal and immune sera. The incompatibility of human blood groups is a consequence of the presence of hemagglutinins. Hemagglutinins are also formed by some bacteria and may possibly be instrumental in the formation of the thrombi observed in the blood vessels after death from certain of the infectious diseases.

Like the other immunological reactions, agglutination is highly specific, an antiserum agglutinating only the homologous antigen. Unlike the lysins and opsonins, however, the activity is heat-stable and the antibody does not require the cooperation of a heat-labile component. The reaction may be observed microscopically by mixing a suspension of bacteria and diluted antiserum on a slide but is most commonly carried out by mixing the two in 0.5 to 1.0 cc. amounts in small test tubes and observing the formation of a precipitate. In the latter instance, varying dilutions of serum (frequently prepared in geometrical progression by mixing with an equal amount of physiological salt solution, *i.e.*, 1 : 10, 1 : 20, 1 : 40, 1 : 80, etc.) are added to the bacterial suspension and incubated at 37° C. over night or at 55° C. for two hours. The highest dilution showing observable flocculation is taken

[58] Ward and Enders. Quoted by Zinsser, Enders and Fothergill: *Immunity Principles and Application in Medicine and Public Health.* The Macmillan Company. New York. 1939. p. 315.

as the titer of the serum; a serum showing agglutination in a dilution of 1 : 10,000 but not in 1 : 20,000 is said to have an agglutinin titer of 1 : 10,000. Such titers are variable to some degree depending upon the density of the bacterial suspension and other factors; light suspensions, for example, showing only a faint turbidity to the eye will give higher agglutinin titers than heavy suspensions.

Cross Reactions.—Although the agglutination reaction is highly specific, certain cross reactions between closely related bacterial species are frequently observed. Such reactions are attributable not to a lack of immunological specificity but to the immunological heterogeneity of the bacterial cell. As indicated above, the cell is made up of a variety of antigenic components, an "antigenic mosaic." Clearly, then, if the same component is present in each of two species of bacteria, an antiserum prepared against the one will agglutinate the other but generally to a reduced titer, *i.e.*, only in the lower serum dilutions. This sharing of antigenic components and the resulting cross reactions are particularly common among the paratyphoid (*Salmonella*) and dysentery bacilli. The agglutinins responsible for

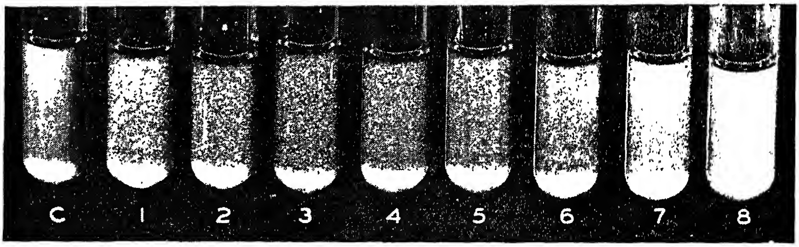


Fig. 31.—The macroscopic agglutination test—flagellar agglutination of *Salmonella*. The control tube, *C*, contains bacterial suspension but no antiserum. Successive dilutions in the numbered tubes are 1:100, 2:200, 1:500, 1:1000, 1:2000, 1:5000, 1:10,000, and 1:20,000. Note agglutination in 1:10,000 but not in 1:20,000.

such cross reactions are sometimes referred to as "group agglutinins" and the phenomenon as "group agglutination."

Agglutinin Absorption.—The direct demonstration of the antigenic heterogeneity of a bacterium and the consequent multiple antibody content of its homologous antiserum are made possible by agglutinin absorption. If a heavy suspension of bacteria is prepared in diluted (commonly 1 : 50) antiserum, incubated for two to three hours, and centrifuged, the supernatant diluted serum will be found to have lost its ability to agglutinate the bacterium with which it was absorbed; the agglutinins for that microorganism have been taken up by the bacterial cells, leaving other agglutinins intact. In practice it is necessary to absorb two or three times and, since the phenomenon is an adsorption, it is not always possible to remove completely the agglutinins in question.

This preferential absorption makes possible the breakdown of the antigenic mosaic of the bacterial cell into component parts. For example, an antiserum prepared against a bacterium containing antigens *A*, *B* and *C*

will contain antibodies *a*, *b* and *c*. If such an antiserum is absorbed by a second bacterium which contains antigens *B* and *C*, antibodies *b* and *c* will be removed leaving *a* intact, and the antiserum will still agglutinate its homologous bacterium. Suppose an antiserum is prepared against the second bacterium which will contain antibodies *b* and *c*. If this serum is absorbed by the bacterium containing antigens *A*, *B* and *C*, it will no longer agglutinate its homologous antigen, for all the antibodies will have been removed. Clearly, then, the complete absorption of a known serum by an unknown organism does not indicate that the unknown organism is necessarily immunologically identical with that with which the known serum was prepared; to prove such immunological identity a "mirror absorption" must be carried out, *i.e.*, each antiserum absorbed with each organism.

The agglutination test and agglutinin absorption, although used to some extent in the diagnosis of infectious disease (as in the Widal test in which typhoid bacilli are mixed with patient's serum), have been found particularly valuable in the study of the relation of bacterial species to one another and in some instances, as in the case of the *Salmonella* (Chapter 19), complex antigenic formulae have been worked out. The determination of the components of the antigenic mosaic of a bacterial species is termed *antigenic analysis*.

The Prozone Phenomenon.—It is not infrequently observed that immune sera showing high agglutinin titers fail to agglutinate the homologous bacteria in low dilutions, *i.e.*, 1 : 100 or less. This portion of the dilution range is designated as the *prozone* or proagglutinoid zone. Prozones, although occasionally observed with fresh sera, are more common and extend over a wider range with old or heated sera. Heating or aging presumably alters the physical state of the antibody in such a way that after adsorption on the bacterial cells it acts as a protective colloid and prevents clumping. That some such alteration has occurred is indicated by the differential filterability of agglutinin and *agglutinoid;* agglutinin is filterable through Berkefeld filters, while agglutinoid is held back.

"H" and "O" Agglutination.—A number of bacterial species, particularly *Salmonella*, *Proteus* and certain others, may agglutinate in one of two ways. Macroscopically the "H" agglutination gives rise to a loose, flocculent precipitate, and upon microscopic examination it may be observed that the bacterial clumps are loose, the flagella of the microorganisms being entangled with one another. The "O" agglutination, on the other hand, produces a finely granular precipitate in which the individual bacterial cells are closely packed together. These types of agglutination have been shown in micromotion pictures by Pijper.[59] They are a consequence of the presence or absence of the flagellar, heat labile "H" antigen. Immunization with the whole bacterial cell containing both flagellar and somatic antigenic components gives rise to antisera containing both types of antibody, the "H" generally in high titer while the "O" antibody is commonly active in dilutions of less than 1 : 1000.

Spontaneous Agglutination.—Some strains of bacteria do not form stable suspensions and are said to be spontaneously agglutinable. This behavior

[59] Pijper: Jour. Bact., 1941, *42*:395.

is particularly characteristic of rough variants and, although not an immunological phenomenon, is frequently of practical importance in agglutination studies.

The Mechanism of Agglutination.[60]—The clumping of bacteria under the influence of immune serum may be taken as evidence *per se* that a force attracting the cells to one another is operative at least at times. Similarly, the fact that bacteria are not in a constant state of agglutination is indicative of a force which tends to hold the cells apart from one another. The attractive or cohesive force is probably that of surface tension, *i.e.*, the

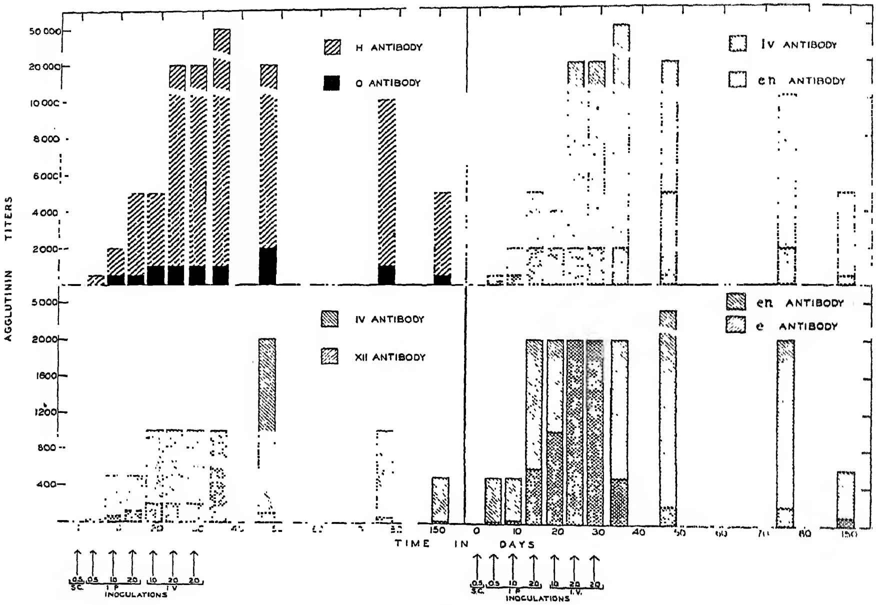


Fig. 32.—The immune response in the rabbit to the antigenic complex of *Salmonella brandenburg.* Upper left, H and O agglutinin titers, showing the usual marked discrepancy between the two. Lower left, the differential response to the two components of the O antigen. Upper and lower right, the response to the components of the H antigen. Note the independent behavior of the antibodies as indicated by variation in peak titers. All titers determined by direct agglutination of heterologous antigens without agglutinin absorption.

interfacial tension at the cell surface, and the repulsion that of like electrical charges, for, as pointed out previously, bacteria are negatively charged at pH's compatible with viability. On the basis of such reasoning it would appear that the balance between these opposing forces determines whether the microorganisms will form a stable suspension or whether they will clump together and settle out.

It was early observed[61] that the presence of an electrolyte is essential to

[60] For the literature to 1919 see Buchanan: Jour. Bact., 1919, *4*:73.

[61] Bordet: Ann. Inst. Pasteur, 1899, *13*:225.

agglutination; if both immune serum and bacterial suspension are dialyzed free of salt before mixing, the cells are not agglutinated, but if a trace of salt is added to the mixture, agglutination takes place at once. This behavior, it will be seen, corresponds to that of a mixture of two colloids of opposite charge, such as gum mastic and gelatin, when the one is added in too small an amount to precipitate in the absence of salt. That the repelling effect of electrical charge is an important factor is also indicated by the agglutination of bacteria in the absence of antibody when the *p*H is lowered to the isoelectric point of the cells, a phenomenon termed *acid agglutination.* It was formerly thought that bacterial species might be sharply differentiated from one another on the basis of the *p*H of their acid agglutination, but this has not proved to be true.[62]

The agglutination of bacterial cells is, however, a function not only of electrical charge on the cells but also of the cohesive forces tending to draw them together. The studies of Northrup and DeKruif[63] in which both potential difference (between the cells and the suspending medium) and cohesive force were directly measured have shed considerable light on the mechanism of agglutination. These workers found that electrolytes in low concentration (0.01 N) affect primarily the potential, and in high concentration also decrease the cohesive force. If the cohesive force is not affected, agglutination occurs when the potential is reduced below the critical point of 15 millivolts. If, however, the cohesive force is decreased, the critical potential is also decreased and, therefore, in concentrated salt solutions agglutination does not take place even though there is no measurable potential. Immune serum, presumably adsorbed on the surface of the bacterial cells, while reducing the charge somewhat, appears to function by preventing the salt from decreasing the cohesive force, and agglutination occurs at the critical point of 15 millivolts. Furthermore, if the potential difference is reduced by electrolyte to 15 millivolts or less in a bacterial suspension, the addition of immune serum raises the reduced cohesive force and agglutination takes place. Examination of typhoid bacilli by the electron microscope in the presence of immune serum has shown that the flagella become thickened by the deposition of an antibody film approximately 21 Å. thick and the cell walls become more opaque and less definite in outline. The serum-sensitized surfaces appear to be sticky, not only for one another, but also for other particulate matter.[64]

Precipitins.—The mixture of an immune serum prepared against a soluble antigen, such as egg albumin, with its homologous antigen results in the formation of a precipitate. This phenomenon is termed the *precipitin reaction* and the antibody a *precipitin;* the antigen is sometimes designated as a *precipitinogen.* The physical state of the antigen used for immunization is not of particular importance; antibacterial sera, for example, will give precipitates when mixed with preparations of the soluble cell substance of the microorganisms, and precipitins may almost always be demonstrated in lytic, antitoxic, opsonic and agglutinating antisera. It has been found in

[62] For a summary of the literature see Gouwens: Jour. Inf. Dis., 1923, *33*:113.
[63] Northrup and DeKruif: Jour. Gen. Physiol., 1922, *4*:639, 655.
[64] Mudd and Anderson: Jour. Immunol., 1941, *42*:251.

the first instance that the precipitate will fix complement and the flocculation of toxin-antitoxin mixtures has been referred to above. Like agglutination, precipitation does not occur in the absence of electrolyte and does not require the presence of heat-labile substances such as complement.

In contrast to the agglutination test, the precipitin test is carried out with undiluted, or only slightly diluted, serum but the antigen solution is diluted in series. The reagents may be mixed in the usual way, or the antiserum may be pipetted into very small test tubes and the solutions of diluted antigen carefully layered on top with no mixing. In the first instance a precipitate is formed which settles out like agglutinated bacteria, while in the second a precipitate forms at the interface between serum and antigen solution. The latter test is termed a "ring test." The end-point of either test may be taken as the highest antigen dilution with which the serum forms an observable precipitate and the titer of the serum given in these terms. Potent antisera may have extremely high titers, precipitating antigen in dilutions of 1 : 100,000 to 1 : 5,000,000. A more accurate measure of the precipitin content of an antiserum, however, is that of the antigen dilution in which precipitation first occurs (and generally is heaviest), for it is at this point, sometimes termed the *equivalence zone*, that the antigen-antibody ratio is optimal for precipitation. Two such optimal ratios may be distinguished, one when the antigen is held constant and the antibody added in increasing amounts and the other when the antibody is constant and antigen is added. The two are not necessarily identical, as might be expected, although very nearly so in the Ramon flocculation, in which the end-point is clearly that of optimum antigen-antibody ratio. In other instances the amount of antibody required for most rapid flocculation when the antigen is kept constant may be six to eight times as much as when the antibody is kept constant. The explanation of this phenomenon lies in the variable proportions of antigen and antibody which make up the precipitate, as will appear in a later section.

The precipitate formed consists of antigen and antibody in variable proportions depending upon the relative amounts of the reacting substances. The antigen is diluted rather than the antiserum because relatively large amounts of antibody are required. As in the agglutination reaction, antibody is adsorbed on to the surface of the antigen, but, since the antigen molecules are much smaller than the bacterial cells, the surface to be covered is proportionally greater and consequently large amounts of antibody are required. It follows, of course, that the proportion of antibody to antigen is much greater in the precipitate formed than in agglutinated bacteria.[64a] The mechanism of the precipitin reaction is probably essentially that of agglutination, molecules instead of bacteria being clumped. It will be discussed at greater length in a later section.

The close relation between the precipitation and agglutination reactions is indicated by the agglutination by a precipitating antiserum of particles of inert material, such as collodion, upon which the antigen has been adsorbed. These particles then behave as particles of antigen and may be set

[64a] *Cf.* Zinsser: Jour. Immunol., 1930, *18*:483.

up with serial dilutions of antiserum as in the agglutination test.[65] Goodner[66] has carried out the reaction in the reverse by adding collodion particles to the antigen-antibody mixture, the complex being adsorbed and the particles agglutinated; he has called this "collodion fixation."

The precipitin reaction occurs not only between antibody and complete antigen but also with some partial antigens or haptenes. An antipneumococcus serum, for example, will react not only with a solution of the pneumococcus cell substance but also with pure capsular polysaccharide. When the haptene is of high molecular weight, as in the case of the polysaccharides, a visible precipitate is formed, but when it is a simple compound such as an organic acid or monosaccharide no observable precipitate appears. The reaction between antibody and such haptenes may, however, be shown by the fact that antisera treated with haptene will no longer precipitate the complete antigen; the antibody has already reacted with the partial antigen. The development of knowledge of the haptenes has made possible quantitative studies on the antigen-antibody reactions; when both reacting substances are proteins the two cannot be distinguished by chemical methods in a precipitate, but if the one be a polysaccharide and the other a protein, the reacting substances are readily differentiated and determined by ordinary analytical methods.

The precipitin reaction, like the other immunological reactions, is highly specific, but cross reactions are observed between chemically similar antigens that are analogous to "group agglutination." Such cross reactions occur between antisera for the blood proteins of man, chimpanzees, gorillas and various species of monkeys. An antihuman serum prepared by immunizing a monkey, however, does not show cross reaction with that monkey's serum. Other immunological relationships, such as those between the caseins of various animal species, the egg albumins, etc., appear in the precipitin reaction also. In most instances, however, the differentiation is sharp, or may be made so by the use of slightly diluted sera, and the simplicity and accuracy of the precipitin test has resulted in its frequent use in forensic medicine. The differentiation between chicken blood and human blood by the precipitin test in a case of suspected murder, for example, has the highest standing as evidence in court.

The studies of Landsteiner and others on the chemical basis of the specificity of antigens, which have already been discussed, have made use of the precipitin reaction almost entirely, and the similarities of molecular structure of antigens or the immunologically determinant groups provide a sound basis for the understanding of cross reactions.

Ablastin.—The existence of an antibody which inhibits the reproduction of an invading microorganism was postulated by Ascoli[67] on the basis of his experiments with the anthrax bacillus. Similar results were reported later by Dochez and Avery[68] for the pneumococcus which suggested that

[65] Cannon and Marshall: Jour. Immunol., 1940, *38*:365.
[66] Goodner: Science, 1941, *94*:241.
[67] Ascoli: Ztschr. f. physiol. Chem., 1906, *48*:220; Centralbl. f. Bakt., Abt. II. 1908, *46*:178.
[68] Dochez and Avery: Jour. Exp. Med., 1916, *23*:61.

immune serum temporarily inhibited the multiplication of these organisms and depressed their proteolytic and glycolytic activities. This effect was attributed to "antiblastic" or reproduction-inhibiting antibodies present in the immune serum. In reported experiments with bacteria, however, the results may be interpreted in other ways, and the existence of an ablastin for bacteria has not been conclusively demonstrated.[69]

A reproduction-inhibiting antibody developed in response to invasion with certain of the parasitic protozoa, trypanosomes, was independently discovered by Taliaferro.[70] In this case the activity of the antibody was sharply differentiated from that of coexisting lysin and was demonstrable directly by cessation of cell division and indirectly in terms of variability in size of the microorganisms, *i. e.*, in a population of rapidly reproducing organisms the size of the individual cells is subject to wide variation whereas size is relatively constant in a population consisting entirely of adult forms. It is not necessary, however, to postulate the existence of an antibody which specifically inhibits reproduction for the consequences of the union of antibody with the antigen of the living microorganism may well be such as to inhibit normal physiological functions including those associated with growth and cell division. For example, *Nippostrongylus* larvae in and about which immune precipitates have been formed are stunted and immobilized.

Perhaps related to ablastins are the "non-absorbable" antibodies which have been demonstrated in certain worm infections. Campbell[71] has found that antibodies to the larval tapeworm, *Cysticercus crassicollis*, present during the later stages of infection, cannot be absorbed from the serum by treatment with the parasites. He regards these nonabsorbable protective substances as antienzymes, whose presence interferes with the processes of growth and reproduction, but which are not absorbed because the antigen is elaborated only by actively metabolizing cells and is not present in those which are physiologically quiescent. Possibly to be regarded in the same light is the precipitin present in convalescent yellow fever serum which reacts with yellow fever serum taken during the acute stage of the disease but not with the virus.[72] This phenomenon, however, has been interpreted by some as indicative of the presence of a "pathological protein" in yellow fever. Avery and his co-workers[73] have observed a similar protein which appeared in the blood of human beings and of monkeys in various etiologically unrelated infections, and which formed a precipitate with the C substance (somatic polysaccharide) of pneumococci. Serum showing this activity has been called "acute phase serum."

Neutralizing Antibodies.—In the light of the capacity of an immune serum to lyse, kill or sensitize bacteria to phagocytosis, it follows that the injection of a suspension of pathogenic bacteria in homologous immune serum may have less serious consequences to the susceptible animal than

[69] *Cf.* Blake: Jour. Exp. Med., 1917, *26:*563; and Bordet: Compt. Rend. Soc. de Biol., 1931, *108:*752.

[70] *Cf.* Taliaferro: Amer. Jour. Hyg., 1932, *16:*32.

[71] Campbell: Jour. Immunol., 1938, *35:*195, 205, 465; Jour. Inf. Dis., 1939, *65:*12.

[72] Hughes: Jour. Immunol., 1933, *25:*275.

[73] Avery, Abernathy and MacLeod: Jour. Exp. Med., 1941, *73:*173 *et seq.* See also Löfström: Brit. Jour. Exp. Path., 1944, *25:*21.

the inoculation of the bacteria alone. Since the usual serological reactions may be carried out with the filterable viruses only at considerable inconvenience owing to difficulties in the preparation of the antigen and the like (p. 798), it has become customary to evaluate the antibody content of antiviral sera in terms of their neutralizing capacities, *i. e.*, ability to prevent infection when injected with the infectious material. This technique is, however, not confined to the filterable viruses, for the "protective titers" of certain antibacterial sera, as, for example, in studies on the efficacy of typhoid immunization (p. 410), have been found a more satisfactory measure of antibody content than the measurement of agglutinin, precipitin or other single antibody titers. It is becoming more and more generally used as a method of assay of the potency of antisera.[74]

In practice, varying amounts of immune serum are mixed with a constant amount of bacterial suspension or infectious material and injected into susceptible animals either with or without a period of preliminary incubation. In this way a crude approximation of the neutralizing or protective capacity of an immune serum can be made.

Whether the "virus neutralizing" or "protective" capacity of an immune serum can be accounted for on the basis of the combined activity of the other known antibodies or whether other factors are involved is open to question. Since, however, the differentiation of the kinds of antibodies from one another is largely a matter of the technique by which the antigen-antibody reaction is demonstrated, as will appear, the neutralizing or protective action of an immune serum which is not identical with precipitin, agglutinin or other antibody is legitimately regarded as a separate antibody.

The Nature of Antibodies.—The question of the nature of the antibodies is one of no small practical as well as theoretical importance. These substances are intimately associated, if not identical, with the globulin fraction of the serum proteins and may be separated from the other serum constituents, sometimes by simple dilution with distilled water but more commonly by some salting out procedure. With one or two exceptions, the range of salt concentration over which they are precipitated is broad and not sharply defined. Attempts to further differentiate the active fraction into euglobulin and pseudoglobulin have not been successful, the antibody appearing sometimes in the one, sometimes in the other and not infrequently in both, depending upon the animal immunized. Electrophoretic fraction of serum globulin in the Tiselius apparatus, however, allows the separation of three components on the basis of mobility. These are designated α-globulin, β-globulin and γ-globulin in order of decreasing mobility. Antibody appears to be associated almost exclusively with the γ-globulin fraction, though β-globulin may occasionally show some activity. Probably the purest antibody preparations have been those obtained by dissociating antigen-antibody precipitates, the antibody having been previously purified by salting out; about 0.01 mg. of globulin per unit of diphtheria antitoxin is precipitated by toxin. Northrup[75] has prepared crystalline diphtheria antitoxin which appears to be a pure protein, crystallizing in thin

[74] *Cf.* Zittle: Jour. Immunol., 1943, *47*:97.

[75] Northrup: Proc. Amer. Phil. Soc., 1941, *85*:13.

plates and containing 700,000 to 1,000,000 antitoxic units per gram. It has not been possible as yet, however, to separate antibody from serum globulin and, in spite of older reports of the preparation of non-protein antibody solutions, present evidence justifies the current belief that *antibodies are modified serum globulin.* The physical and chemical properties of antibodies are, then, those of globulin. More precise knowledge is obviously directly dependent upon the state of knowledge of protein chemistry, whose present unsatisfactory nature is indicated by the feeling on the part of a number of competent investigators that the serum proteins, including globulin, do not exist as such in the body but are artifacts arising as a consequence of laboratory manipulation.

If antibodies are to be regarded as modified serum globulin, the nature of the modification, *i. e.*, the differences between immune and normal serum globulin, is of considerable interest. Unfortunately these differences are expressed chiefly in the ability of the modified globulin to combine with the antigen, for, in spite of intensive study, there appear to be no consistent differences in physical and chemical properties between the two (see p. 282). Exception must be made in the case of resistance to the action of proteolytic enzymes; the immune globulin appears to be considerably the more resistant to enzymatic hydrolysis.[76]

Antibody Formation.—The Breinl-Haurowitz theory[77] of antibody formation postulates that some antigen remains within the antibody-forming cell to provide a template or pattern for the synthesis of specific antibody. On this basis Pauling[78] proposed, on purely hypothetical grounds, a theory of the structure and process of formation of antibody. He has suggested that if the normal globulin molecule is formed by the coiling of peptide chains after their synthesis, the surface character of the molecule could be determined in the process of coiling. In the presence of the antigen template the peptide chain is coiled in such a way as to give a surface pattern of electronic van der Waals forces complementary to the pattern of the determinant group of the antigen molecule (see p. 282). Dissociation of the antigen-antibody complex frees the antigen to act as a template for the formation of another antibody molecule, and so on. Immune globulin would, therefore, differ from normal globulin only with respect to the manner of coiling of the peptide chains.

This theory is susceptible to test for normal globulin may be denatured slowly by heat, alkali, etc., to uncoil the peptide chains; then if it is renatured in the presence of the antigen, the recoiling should be oriented with respect to the antigen. This kind of experiment has been carried out by Pauling and Campbell[79] who denatured ox γ-globulin by heating to 57° C. for eleven to fourteen days in the presence of 1,3-dihydroxy 2,-4,6-tri(*p*-azophenylarsonic acid) benzene or pneumococcus Type III polysaccharide. After removal of the "antigen," the globulins gave specific

[76] This has been made the basis of a method of antibody purification by partial enzymatic digestion of immune serum patented by Parfentjev: U. S. Patent Office, Application Oct. 9, 1934, Serial No. 747532, Patented, Dec. 22, 1936, *2065*:196.

[77] Breinl and Haurowitz: Ztschr. f. physiol. Chem., 1930, *192*:45.

[78] Pauling: Jour. Amer. Chem. Soc., 1940, *62*:2643.

[79] Pauling and Campbell: Jour. Exp. Med., 1942, *76*:211.

precipitin reactions and in the second instance specifically agglutinated Type III pneumococcus but only in very low dilution. These observations provide strong support for the theory and are especially notable because they are the first demonstration of antibody formation *in vitro.*

Consistent with the intimate relation of antibody and serum globulin is the increase in serum globulin, especially γ-globulin, on immunization. This increase in general appears to take place at the expense of serum albumin, which is decreased, but in some cases the reduction in albumin does not occur and the total serum protein increases to as much as 15 per cent. Not all of the new globulin is antibody, however, for less than one-half is capable of reacting with antigen. It is of some interest that large and small antibody molecules are produced, depending upon the animal species immunized; those from the horse, cow and pig are large with molecular weights of 910,000 to 930,000, while those formed by man, monkey and rabbit are considerably smaller with molecular weights of 156,000 to 196,000.[80]

The "Unitarian" Hypothesis.—It was originally supposed by Ehrlich that the various demonstrable antibodies—antitoxins, agglutinins, precipitins and the like—are separate, distinct and independent substances, a concept that is apparently supported by discrepancies in the titer of one antibody and that of another in a given immune serum. The essential similarity of the agglutination and precipitation reactions was, however, early apparent, and with increasing knowledge it has become clear not only that it is unnecessary to postulate a multiplicity of antibodies but that the weight of experimental evidence suggests that a pure antigen stimulates the formation of but a single antibody. This essential identity of the various antibodies has been urged by a number of workers, and by Zinsser[81] in particular, in what is known as the *unitarian hypothesis.*

The basic feature common to all the immunological reactions is the union of antigen and antibody and, according to the unitarian hypothesis, this antibody is the same regardless of the consequences of union, which are, of course, variable and dependent upon the nature of the antigen and the conditions under which the reaction takes place. After union with antibody the antigen is sensitized to the lytic action of complement, is able to fix complement, is rendered susceptible to phagocytosis or is agglutinated or precipitated. The outcome is a consequence of the physical state of the antigen, *i.e.*, whether it is cellular, particulate or in solution, and of the particular consequences of union the experiment is designed to show, *i.e.*, whether the test is one of complement fixation, agglutination, etc. Instances of this have already been noted, such as the precipitation of toxin by antitoxin in the Ramon flocculation, the agglutination of antigen-coated particles by precipitating sera, the fixation of complement by precipitates from the precipitin reaction and hemagglutination in the absence of complement.

The unitarian concept postulates only that each antigen gives rise to a single antibody; a multiple antigen such as a bacterial cell will, of course,

[80] *Cf.* Kabat: Jour. Exp. Med., 1939, *69:*103.
[81] *Cf.* Zinsser: Jour. Immunol., 1921, *6:*289.

stimulate the formation of a series of antibodies, one for each of the immunologically active constituents of the cell. Discrepancies in antibody titer of antibacterial sera may, then, appear without invalidating the concept of the unity of antibodies; the flagellar agglutinins for the typhoid bacillus, for example, may have quite a different titer from that of precipitins for the cell substance. Similar discrepancies in complement-fixing and precipitin titers of antisera for pure antigens, such as egg albumin, in which only a single antibody is concerned, are explained by the fact that antigen-antibody ratios optimal for complement fixation are not optimal for precipitation.

The evidence for the essential identity of what were at first regarded as distinct antibodies for a single antigen is indeed impressive, and the unitarian hypothesis may be regarded as substantially in accord with the facts.

THE ANTIGEN-ANTIBODY REACTION

The mechanism of the antigen-antibody reaction has already been touched upon briefly in the preceding sections. The reaction between these two substances may be considered here, however, at somewhat more length and in general terms.

It will already be apparent that the antigen-antibody reaction appears to take place in two separate and distinct stages, the first in which the two combine and the second in which the consequences of that union appear as agglutination, precipitation and the like. In mixtures the two processes undoubtedly go on at the same time; it is not necessary that the first proceed to completion before the second may be initiated. Direct evidence of such a two-step reaction is found in the union of antibody with simple haptenes, in which the former is saturated and unable to react further with complete antigen even though no second stage is observable. The theoretical aspects of this union are concerned not only with the actual mechanism of union but also with the attributes of antigen and antibody that make possible their reaction with one another.

The only comprehensive formulation of the theoretical aspects of immunology is that of Ehrlich,[82] a formulation which has constituted the theoretical basis upon which a vast amount of experimental work has rested. Let us consider Ehrlich's basic concept, first as it was developed, and second in the light of present knowledge.

Ehrlich's Receptor Theory.—Ehrlich's attempt to develop a theoretical basis for the explanation of the immunological phenomena has been touched upon briefly in connection with the antitoxins. The theory he developed, however, is not confined to the toxin-antitoxin reaction but is a general one of immunity in the broad sense.

The receptor theory rests upon the basic assumption that the various cells of the animal body obtain their nutriment from the blood or lymph in which they are bathed through the agency of localized cell substances, the *cell receptors*, which have combining affinities with food substances. The receptors may be regarded as bearing the same relation to the main body of the cell (*Leistungskern*) that the side chains of complex molecules bear

[82] Ehrlich: *Studies on Immunity*. New York. 1906.

to the central molecular nucleus; hence the receptor theory is sometimes known as the *side chain theory*. These receptors may be of simple constitution, adapted to the taking up of relatively simple substances, or they may be highly complex and capable of anchoring large and complex protein molecules. Each cell may, of course, contain a large number of receptors of different affinities and degrees of complexity.

It is plausible to assume that when bacteria or other alien cells or their products are introduced into the body, the combining affinities of certain receptors may be satisfied by bacterial substances just as by similarly constituted food molecules. The anchoring of toxic substances, however, unlike that of food substances, is followed by damage to the cell and loss of the particular side chain or receptor that unites with the toxic element. When injury to the main body of the cell is not carried too far, repair can take place and the receptors be regenerated. In keeping with the principle

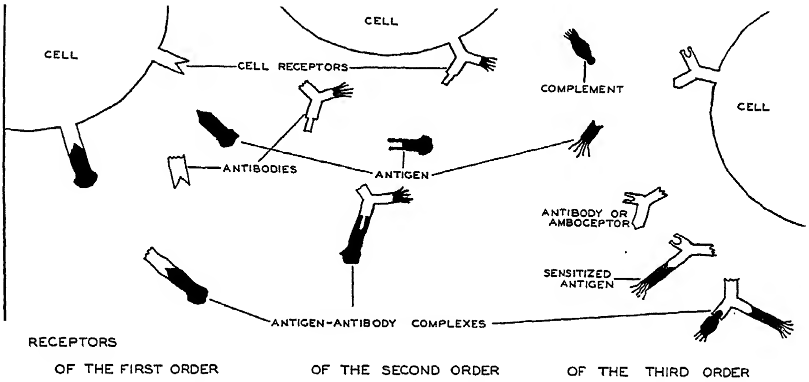


Fig. 33.—Diagrammatic representation of Ehrlich's receptor theory.

enunciated by Weigert regarding the regeneration of tissue cells, there is a tendency in the regeneration of lost parts, in this case receptors, to overcompensate, and the free receptors formed in excess of the needs of the cell are discharged into the blood stream. *These free receptors are the antibodies*, the antitoxins, agglutinins, lysins and the like. They differ in complexity. The simplest, or receptors of the first order (see Fig. 33), are those which combine with toxin and, when free, constitute antitoxin; receptors of the second order are somewhat more complex and are functional in agglutination and precipitation; receptors of the third order are still more complex and function in the lytic reactions in which complement plays a part.

Similar representations of the antigenic substances and of complement necessarily follow in this concept of the nature of the immune bodies. Toxin, for example, is assumed to be a relatively simple substance with two functional parts, a haptophore which unites with the receptor and a toxophore which exerts the poisonous effect of the substance. In this termi-

nology, toxoid would be regarded as toxin in which the toxophore portion is inactivated or destroyed.

The mechanism of the reaction of the antibodies with their respective antigens is apparent from the foregoing considerations. The union of toxin and antitoxin is the simplest type, the haptophore group uniting specifically with the corresponding portion of the free receptor or antitoxin molecule and the toxophore group being neutralized in that it is prevented from exerting its action on the cell. Agglutination is much the same so far as the union of antigen and antibody is concerned, but the presence of the zymophore group brings about clumping. In the case of complement fixation it is assumed that the receptor of the third order, after breaking off as indicated in Fig. 33, functions as a sort of bridge or intermediary body (*Zwischenkörper*) which unites with complement at one end and with antigen at the other, thus sensitizing the antigen to the lytic action of complement.

It must be emphasized that the graphic representations of the structure of the receptors and antigens were never intended to be other than purely imaginary pictures constructed in an attempt to convey a concept difficult to put into words. The small child's definition of a cartoon as "a picture of an idea" may be aptly applied.

It will be clear that, in its simplest terms, the receptor theory is a plausible explanation of immunological phenomena. There is evidence, for example, that the various bacterial toxins are bound in each case to particular cells of the organism. The tetanus toxin, when mixed *in vitro* with emulsions of fresh organs, manifests an affinity for different organs in different animals. In man, the horse and the guinea pig only the central nervous system is able to bind the toxin, a finding in complete accord with the pathology of tetanus. If a mixture of tetanus toxin and guinea-pig-brain emulsion in suitable proportions is injected into a susceptible animal, the animal is entirely unaffected, just as if tetanus antitoxin (free receptors) had been used in place of fresh cell substance (cell receptors). Other phenomena are equally well explained in terms of this theory. It will be recalled, for example, that complement was early separated into two parts, mid-piece and end-piece, a finding in keeping with the postulation of zymophore and haptophore portions of this substance.

Certain inadequacies of the receptor theory were apparent almost from the beginning, however, since new knowledge, rather than being predicted by the theory, necessitated various modifications. These discrepancies arose, for the most part, in connection with the mechanism of the first stage of the antigen-antibody reaction and, to some extent, with that of the second stage. It will be recalled that the toxin-antitoxin reaction, for example, was regarded by Ehrlich as analogous to the neutralization of a strong acid by a strong base, a concept that was brought into harmony with experimental observation through the postulation of components of toxin of varying degrees of avidity for antitoxin. Similar modifications and additions, which cannot be considered in detail here, resulted in the course of time in an unwieldy structure that became obviously unsatisfactory.

It may be noted here that the concept of Arrhenius and Madsen of the antigen-antibody reaction as analogous to the neutralization of a weak acid

by a weak base was, in essence, an attempt to retain much of the receptor theory and at the same time account for the Danysz phenomenon and related observations as well as the dissociation of antigen-antibody complexes.

The Antigen-Antibody Reaction as an Adsorption Phenomenon.—In many respects the antigen and antibody solutions are best regarded as colloidal systems and the union of these substances as adsorption phenomena, physical in nature rather than chemical. This interpretation has been strongly urged by Bordet[83] and others. Stripped to its essentials, such an interpretation means that the Freundlich isotherm[84] describes the antigen-antibody reaction with a reasonable degree of accuracy. The successful application of this concept to the first stage of the antigen-antibody reaction is well illustrated in the case of the toxin-antitoxin reaction which has been discussed in this connection in an earlier section. Similarly, the clumping of bacteria under the influence of immune serum, an example of the second stage of the reaction, responds equally well to such an interpretation, as has been pointed out before.

The course of events in the antigen-antibody reaction, as seen from this point of view, may be summarized briefly: there is first a specific adsorption of the immune globulin on the surface of the antigen. The cell or protein molecule thus sensitized behaves, not as the unaltered antigen, but as a particle of serum globulin. The aggregation of the coated particles in the precipitin or agglutination reactions is, then, nothing more than the precipitation of this altered colloid by salt. The sensitized antigen is also capable of adsorbing complement whose fixation may or may not be followed by lysis as the nature of the antigen permits. The evidence for some such mechanism is very strong indeed, and it is highly probable that certain phases of the antigen-antibody reactions are essentially manifestations of the properties of colloidal systems. This inescapable conclusion led many to abandon the receptor theory as entirely outmoded.[85]

This description of the immune phenomena is by no means entirely satisfactory, however, its most glaring deficiency being its inability to account for specificity. While the union of antigen and antibody is, in all probability, an adsorption reaction, why typhoid antibody is adsorbed on the typhoid bacillus and at the same time not on the dysentery bacillus is unexplained. In addition to this failure to account for immunological specificity, certain minor discrepancies may be observed; there is, for example, agglutination or precipitation over a relatively wide *p*H range (in the presence of electrolyte) instead of a marked optimum at the isoelectric point of serum globulin, as might be expected. The adsorption isotherm, furthermore, does not approach an asymptote, and the antigen-antibody reaction

[83] Bordet: *L'Immunité*. Paris. 1920.

[84] The relation between the amount of a substance adsorbed and that remaining in solution was given by Freundlich as $x\ (x/m)\ m = aC^n$, when x is the amount adsorbed, m the surface on which it is adsorbed, C the final concentration of the substance in solution and a and n are constants.

[85] *Cf.* Manwaring: Jour. Immunol., 1926, *12*:177; also a chapter by the same writer in Jordan and Falk: *Newer Knowledge of Bacteriology and Immunology*. University Chicago Press. Chicago. 1928.

shows wide variations from expected values at high concentrations of either reagent. These and other minor points are, however, probably of no great significance. In general, it may be said that although this adsorption theory is thoroughly satisfactory in many respects, it accounts only for some, by no means all, of the immunological phenomena.

The Modern Concept.—As might be expected, the modern concept of the mechanism of the antigen-antibody reaction is an outgrowth of the theories of Ehrlich and Bordet. The reacting substances are regarded as colloids whose union lies in the realm of surface chemistry and is in part physical and in part chemical. For purely technical reasons, the observations which have led to this concept have been made on the precipitin reaction for the most part; a discussion of the present position is, then, best carried on in terms of this reaction.

It is already apparent from the earlier discussion of antigens that immunological specificity may be conferred by a relatively small prosthetic group or haptene attached to the large protein molecule. Furthermore, it was pointed out that similarities in the structure and configuration of these determinant groups are causally related to the immunological behavior of synthetic antigens. Immunological behavior means, of course, the ability both to provoke the formation of antibodies and to react with them. The significance of these observations on immunological specificity to the nature of the antigen-antibody reaction will be apparent at this point; the molecular structure of the prosthetic group determines whether or not the antigen and antibody will react.

There is good experimental evidence, too detailed and extensive to be considered here,[86] that the union of antigen and antibody is an adsorption in which a particular molecular structure is adsorbed with a high degree of specificity. It is probable that there is no chemical union (many of the determinant groups are not characterized by chemically active radicles) but that the adsorption is a consequence of the operation of intermolecular forces (secondary valencies) whose specificity lies in the nature of the field of force arising from the arrangement of the atoms within the determinant group. It is to be noted that the functional structure of the antigen is directly known; that a corresponding field of force, of such spatial distribution that corresponding active points of antigen and antibody can come into apposition simultaneously, is possessed by the antibody is indirectly established by the fact that the two combine and by the structure of the determinant groups of antigens which show cross-reactions. It may be pointed out here that radicles that are mutually replaceable in crystals are immunologically equivalent. The formation of mixed crystals is, however, the severer test, for here all dimensions must fit with a high degree of precision, while in the combination of antigen and antibody only two dimensions need be oriented.

The alteration in normal serum globulin which converts it to antibody is, then, an alteration in the arrangement of polar forces on the surface of the molecule. It is probable that the surface of the normal globulin molecule

[86] *Cf.* Marrack: *The Chemistry of Antigens and Antibodies.* Medical Research Council Special Report Series No. 230. 1939. Also Heidelberger: Bact. Rev. 1939, *3*:49.

presents a mosaic of groups of varying polarity and that some areas are well adapted to binding certain molecules. It is plausible to assume that relatively slight alterations in the structure of the globulin molecule give rise to areas with affinities for considerable areas of certain determinant groups, alterations so slight as to be undetectable by the usual means (see p. 276).

Valence of Antigen and Antibody.—Both antigen and antibody show evidence of heterogeneity with respect to their reaction with one another. A given preparation of an antigen, such as pneumococcus polysaccharide, contains some antigen that will not combine with antibody, and artificial antigens contain only an average number of reactive groups per molecule, some of which may have too few to combine. Similarly, the avidity of anti-

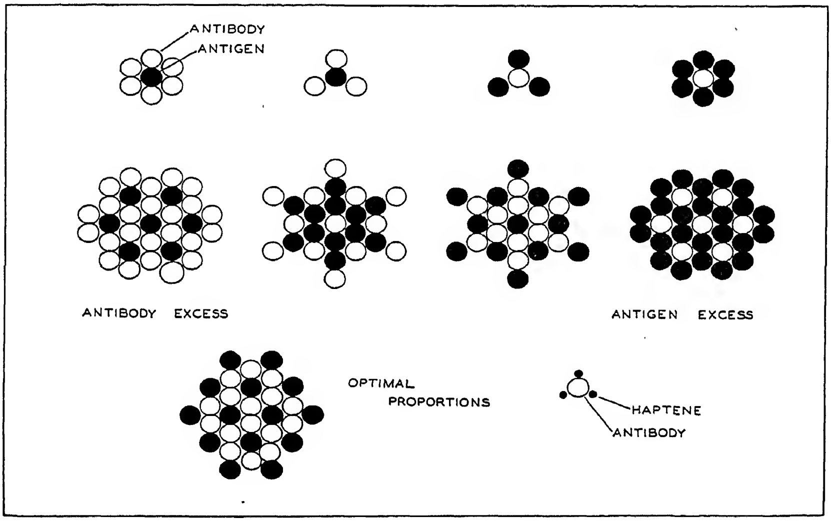


Fig. 34.—Diagrammatic representation of possible arrangements of antigen and antibody molecules in the complex. Modified from Marrack.[86]

body is variable, the more avid molecules combining rapidly with antigen while the less avid ones combine much more slowly. Antigen and antibody may, then, coexist without union if they are so-called modified antigen and weak antibody. It is not definitely known what differences are reflected by this behavior; possibly incompletely developed adsorption sites occur.

The valence of both antigen and antibody has been of some interest, especially in connection with the nature of the second stage of the antigen-antibody reaction. By analysis of antigen-antibody precipitates,[87] for example for arsenic when arsenic-containing artificial antigens are used or for total nitrogen in the case of polysaccharide-antibody complex, it is possible to determine the maximum number of antibody molecules which combine

[87] See the review by Heidelberger: Bact. Rev., 1939, *3*:49.

with a single molecule of antigen. The valence of the antigen appears to be a function of the surface area of the molecule; the functional valence of ovalbumin of a molecular weight of 40,500 is about 5 to 6, while that of thyroglobulin of 650,000 is 30 to 40.[88] These are, of course, minimal values. Maximum values are readily obtained in the case of artificial antigens and it has been found that 10 to 20 active groups must be introduced into the molecule. Not all of such a maximum number of active groups are functional in the reaction with antibody, for when they are too close together the antibody molecules get in one another's way to prevent complete utilization, a phenomenon termed *steric hindrance*.

The valence of the antibody molecule has been the subject of some discussion which cannot be considered at length here. Suffice it to say that, while some workers believe that antibody is regularly univalent, it is generally believed that present evidence indicates that it is usually divalent. Currently accepted theories of antibody formation include the assumption of divalency. Univalent or "incomplete" antibody is known to occur, however, and possibly represents the "weak" antibody noted above.

The Second Stage of the Antigen-Antibody Reaction.—The second stage of the antigen-antibody reaction is generally regarded as an extension of the process of union and therefore specific, rather than a separate and non-specific process. The specific adsorption involves, of course, the apposition of polar groups as indicated above. Thus, if the antibody is divalent it can combine with two molecules of multivalent antigen, the antigen with additional antibody, etc., to form a mesh-like arrangement or lattice structure as illustrated in Fig. 34, large aggregates of which settle out as the antigen-antibody precipitate. This is the *lattice hypothesis* of Marrack.

This theory is dependent, of course, on the existence of at least divalent antibody and it is for this reason that the valency of antibody has been of particular interest. As indicated above, present evidence strongly supports its multivalency. Thus, if increasing amounts of antigen are added to a constant amount of antibody, three zones may be distinguished: first, a zone of antibody excess in which uncombined antibody is present; second, an equivalence zone in which both antigen and antibody are completely precipitated; and, third, a zone of antigen excess in which uncombined antibody is present. The proportions of antigen and antibody in the precipitate vary with the initial concentrations of the reacting substances and behave as if they were multivalent with respect to one another, the observed molecular ratios being consistent with the assumption of divalent antibody. Similarly, the "incomplete" or monovalent antibody described by Heidelberger and Mayer[89] combined with antigen but did not give a precipitate, and Pauling and his co-workers[90] have shown that synthetic antigens containing two or more haptenic groups per molecule would form a precipitate with antibody, while those containing only one haptenic group would not.

[88] Heidelberger: Jour. Amer. Chem. Soc., 1938, *60*:242.
[89] Heidelberger and Mayer: Jour. Bact., 1940, *39*:37.
[90] Pauling, Pressman, Campbell, Ikeda and Ikawa: Jour. Amer. Chem. Soc., 1942, *64*:2994.

Evidence of this sort, of which there is a good deal,[91] strongly supports the lattice hypothesis.

It has been urged by some, especially Eagle[92] and Boyd[93] in recent years, that precipitation is the non-specific result of neutralization or blocking off the polar groups of the complex; if the polar groups remaining on the free surface are not sufficient to keep the complex in solution, it behaves as a hydrophobic colloid and is precipitated by electrolyte. The evidence does not appear to support this view.

The combination of antigen and antibody is regarded by Heidelberger[94] as a chemical reaction between reacting substances which are multivalent with respect to one another and subject to the law of mass action. From this point of view the union may be regarded as taking place in a series of competing bimolecular reactions. The initial reaction between antigen (S) and antibody (A) would be

$$\mathrm{A} + \mathrm{S} \rightleftharpoons \mathrm{AS}$$

In the region of excess antibody the second step would be

$$\mathrm{AS} + \mathrm{A} \rightleftharpoons \mathrm{AS{\cdot}A}$$
$$\mathrm{AS} + \mathrm{AS} \rightleftharpoons \mathrm{AS{\cdot}AS}$$

then

$$\mathrm{AS{\cdot}A} + \mathrm{A} \rightleftharpoons \mathrm{AS{\cdot}A{\cdot}A}$$
$$\mathrm{AS{:}AS} + \mathrm{A} \rightleftharpoons \mathrm{AS{\cdot}AS{\cdot}A}$$
$$\mathrm{AS{\cdot}A} + \mathrm{AS{\cdot}A} \rightleftharpoons \mathrm{ASA{\cdot}ASA}$$
$$\mathrm{AS{\cdot}A} + \mathrm{AS{\cdot}AS} \rightleftharpoons \mathrm{ASA{\cdot}ASAS}$$
$$\mathrm{AS{\cdot}AS} + \mathrm{AS{\cdot}AS} \rightleftharpoons \mathrm{ASAS{\cdot}ASAS}$$

with the formation of particles large enough to settle out of solution as the visible precipitate. In the region of antigen excess the sequence of events can be represented by interchanging A and S in the above expressions. In the equivalence zone the AS formed in the initial reaction would polymerize with the formation of large aggregates with the formula $(\mathrm{AS})_x$.[95] Hershey[96] has developed a descriptive theory of the antigen-antibody reaction, based in part on probability considerations, which allows the calculation of equilibrium constants which agree well with observed values.

The extent to which the indicated mechanism of the precipitin reaction may be generalized to include other manifestations of the antigen-antibody reaction is not yet altogether clear. There are many indications, however, that the process of union is essentially the same in all cases, though final proof is lacking. There is as yet no answer to such questions as that of the manner in which a red blood cell is rendered susceptible to the lytic action of complement or that of the nature of the process of toxin neutralization.

[91] Reviewed by Pauling, Campbell and Pressman: Physiol. Rev., 1943, *23*:203.

[92] Eagle: Jour. Immunol., 1930, *18*:393.

[93] Boyd: Jour. Exp. Med., 1942, *75*:407.

[94] Heidelberger: *Relation of Proteins to Immunity*. Chapter XVII in Schmidt: *Chemistry of the Amino Acids and Proteins*. Charles C Thomas. Springfield, Ill. 1938.

[95] Actually the ratio of antibody to antigen is greater than unity in the equivalence zone, probably always more than 2; hence a more accurate expression is $(\mathrm{A_nS_m})_x$.

[96] Hershey: Jour. Immunol., 1941, *42*:455; *ibid.*, 1942, *45*:39.

The relation of the modern concept of the antigen-antibody reaction to the older theories will be readily apparent. It may be pointed out that Ehrlich's concept of a chemical basis of specificity has been borne out; haptophores and receptors are, in present terminology, determinant radicles and adsorption sites. The mechanism of union, however, conforms to Bordet's adsorption hypothesis, and what were once regarded as mutually exclusive theories have been found to be but two different aspects of the immunological reactions.

CHAPTER 13

IMMUNITY—THE IMMUNE STATE

The foregoing discussion of antigens, antibodies and the antigen-antibody reactions is, essentially, a consideration of the various components of that state of increased specific resistance designated as immunity. The question arises, then, as to what extent these component parts represent the immune state. Can they be put back together to yield a satisfactory account of this phenomenon? In general, it may be said that such a synthesis does result in a relatively complete picture; it is certainly far more satisfactory than corresponding attempts to analyze virulence and nonspecific resistance.

It will be clear from preceding considerations that two kinds of activity, closely interrelated, are operative in immunity; the antibodies, present in the body fluids, function in the first case, and the body cells, the phagocytes, in the second. The two are not infrequently separated, the activity of antibodies constituting the subject matter of *humoral immunity* and that of the phagocytic cells making up the subject of *cellular immunity*.

Humoral Immunity.—Since the appearance of antibodies follows recovery from many of the infectious diseases, it is tempting to assume that these substances are causally related to the observed state of increased resistance. Observation and experimental evidence have amply justified this assumption, obviously in some instances and in a more subtle fashion in others.

An antibody whose association with the immune state is direct and clear is antitoxin. The toxin produced by the invading microorganism has an affinity for the cells as well as antitoxin present in the body fluids, and the protective effect of circulating antibody must, then, be due in part to its superior avidity for the toxin. Such superior avidity generally, but not always, exists. Instances are known in which the toxin unites with the tissue substance in preference to combining with the antitoxin in the blood. The affinity of the cell substance for the toxin is not a constant quality, but fluctuates under different conditions, notably in the upper limits of active immunization. The tissues of an animal treated with increasing doses of toxin sometimes become hypersensitive to the action of the toxin and, in spite of the fact that large quantities of antitoxin are circulating in the blood, the toxin combines by preference with the tissue substance and causes the death of the animal. Variation in the avidity of of circulating antitoxin has been pointed out above. In general, however, the presence of circulating antitoxin is the determining factor; for example, it is not necessary that antibodies to the bacterial cell substance be present in an individual immune to diphtheria or tetanus. The skin tests for immunity, the Schick test for immunity to diphtheria and the Dick test for

immunity to scarlet fever, are tests for the presence of antitoxin; the toxin is injected intradermally and, if not neutralized, gives rise to a local reaction.

The presence of the bactericidal and lytic antibodies is clearly of considerable advantage to the host and these substances are also an obvious part of the defense mechanism. Similarly, the inhibition of multiplication of trypanosomes is an important part of the defense against certain species such as *Trypanosoma lewisi* (p. 715). Agglutinins and precipitins would appear on the surface to serve no useful purpose in the defenses of the immune animal; for example, bacteria are not killed by agglutination. The clumping of bacteria and the precipitation of soluble foreign protein, however, considerably facilitate the process of phagocytosis in that many bacteria may be engulfed at one time and insoluble masses of precipitated foreign protein may thus be removed.[1] In the case of pneumococcus antisera, for example, protective antibody as measured by mouse assay appears to be identical with precipitin and agglutinin. The pneumococcus antibody has been found to enter pneumonic lesions in rats, agglutinating the free pneumococci in the alveoli and thus stopping the spread of the infection.[2] Furthermore, heterologous protection may be demonstrated when portions of the antigenic mosaic are shared.[3] Not all antibodies demonstrable by *in vitro* antigen-antibody reactions have protective qualities, however; in the case of the enteric bacilli protection appears to be associated with antibody to the heat-stable somatic antigens. It may be noted that in one instance there is evidence that precipitins play a direct part in defense; it has been found[4] that the larvae of *Nippostrongylus muris* are stunted and immobilized in the skin of the immune animal with the formation of precipitates in and around them, and a similar reaction may be observed *in vitro*. In general, however, the precipitins and agglutinins function as a part of the defensive scheme in conjunction with the process of phagocytosis.

The importance of phagocytosis in resistance to infection is very great indeed and, in consequence, the opsonins assume particular importance from the theoretical point of view. In practice, though, opsonin titer is generally low as compared to antitoxin and other antibody titers and is highly variable; the value of the opsonic index as a measure of immunity is not great, possibly because of the use of polymorphonuclear leucocytes in *in vitro* tests. Opsonic activity, of course, involves the direct cooperation of certain cellular elements of the body, and any consideration of these antibodies, as well as agglutinins and precipitins, forces consideration of the cellular defenses and indicates the futility of any attempt to differentiate sharply between humoral and cellular immunity.

Cellular Immunity.—The rôle of phagocytic cells in resistance to infection was established by Metchnikoff[5] on the basis of studies on lower forms such as the water flea, *Daphnia*. He distinguished two types of cells,

[1] *Cf.* Cannon: Physiol. Rev., 1940, *20*:89.
[2] Wood: Jour. Exp. Med., 1941, *73*:201.
[3] See the studies on the typhoid bacillus by Luippold: Amer. Jour. Hyg., 1942, *36*:354.
[4] Sarles and Taliaferro: Jour. Inf. Dis., 1936, *59*:207; Taliaferro and Sarles: Jour. Inf. Dis., 1939, *64*:157.
[5] Metchnikoff: *Immunity*. Cambridge. 1905.

the *microphages* or polymorphonuclear leucocytes (heterophils) and the *macrophages* or large mononuclear cells occurring both free in the blood stream and fixed as tissue cells. The former were regarded as of prime importance in the bacterial infections, while the macrophages were thought to be only indirectly active except in chronic infections such as tuberculosis and leprosy. With advances in knowledge, however, it has become clear that although their immediate mobilization accentuates the limited significance of the polymorphonuclear leucocytes, the variety of fixed and free connective tissue cells of mesenchymal origin, grouped under the general head of macrophage, is of considerably greater importance in resistance to disease.

The distribution and interrelationships of these phagocytic cells may be considered briefly in outline form:[6]

A. The predominantly fixed cells of the reticular and loose connective tissue which can be divided into two great groups:

(1) fixed and free macrophages, including the reticular cells, and

(2) the fibroblasts of connective tissue and the endothelial cells lining the ordinary blood vessels.

(1) Macrophage is essentially a physiological designation for almost any large mononuclear connective tissue cell which is predominantly phagocytic and includes

(*a*) fixed cells such as

1. *pericytes* (Maximov), fixed, undifferentiated outstretched cells in the adventitia of all the small blood vessels of loose connective tissue throughout the body.
2. *reticular cells* which, with fibers, form the stroma of all reticular (myeloid and lymphatic) tissues, and
3. *littoral cells* (Siegmund) which line the sinuses of the reticular tissues, liver, hypophysis and adrenal. In the liver these are designated as *Kupffer cells*. Although frequently called endothelial cells or cells of the special endothelium, these are in fact either true reticular cells or have greater developmental potencies than ordinary endothelial cells.

These cells can divide by mitosis, become phagocytic, and develop into fibroblasts or practically any other blood or connective tissue cell. Here it is important that they can become phagocytic either in their fixed position (fixed macrophages) or after rounding up and becoming free (free macrophages).

(*b*) The free cells occurring in the loose connective tissue are variously known as *histiocytes*, *clasmatocytes*, *rhagiocrine cells* or wandering resting cells, are either phagocytic or can become so without morphological change, can reproduce by mitosis, and transform into fibroblasts.

(2) The fibroblasts and endothelial cells are morphologically characterized by outstretched, ill-defined cytoplasm, a large oval vesicular nucleus containing dust-like chromatin granules, and small nucleoli.

(*a*) The fibroblasts divide by mitosis but do not develop into other cells (except in bone and cartilage) and are rarely phagocytic although instrumental in repair and walling off foreign material.

(*b*) The endothelial cells which line the larger blood vessels and capillaries (not including the littoral cells) likewise are rarely phagocytic but may be transformed into fibroblasts.

[6] This material is taken, with only minor modifications, from Taliaferro: *The Immunology of the Parasitic Protozoa.* Chapter XVIII in Calkin's *Protozoa and Biological Research.* Columbia University Press. New York. 1941.

B. The free connective tissue and blood cells.
  (1) The cells of the blood and lymph are classified as to whether of myeloid or lymphoid origin:
    (*a*) The *lymphoid cells* of the blood include the various sized lymphocytes which, together with monocytes, are termed *agranulocytes*. They divide mitotically and can develop into macrophages with all the latter's developmental potencies. As they become transformed into macrophages, they show increased amounts of cytoplasm, their nuclei take on macrophage characteristics and they become phagocytic. These transitional forms are known as *polyblasts*.
    (*b*) The *myeloid cells* are the various *granulocytes* (heterophils or polymorphonuclears, eosinophils and basophils), the erythrocytes and platelets. Of these the heterophils are functional in immunity by virtue of their phagocytic activity but are "end" cells which do not reproduce or develop into other cells.
  (2) The free mesenchymal cells are lymphoid cells indistinguishable from lymphocytes which occur in varying numbers in reticular and loose connective tissue and here act as precursors or "stem" cells of lymphoid and myeloid cells and hence may give rise to macrophages. They are variously termed *lymphocytes*, *hemocytoblasts*, *lymphoblasts*, *myeloblasts* or *monoblasts* according to varying theories of blood formation. Under normal conditions lymphocytes in lymphatic tissue give rise only to lymphocytes and hemocytoblasts in bone marrow only to myeloid cells but under abnormal stimuli they may exhibit their full potencies for development. These stem cells are self-perpetuating but, as noted above, may arise from the fixed mesenchymal cells.

The Systems of Cells.—It will be apparent that the cells primarily associated with defense against invading microorganisms are widely distributed through the body in the blood and lymph, cartilage, bone, reticular (blood-forming) tissue of the myeloid and lymphatic organs and in the loose connective tissue associated with the skin, omentum, liver, lung, etc. It is customary to group the macrophages into so-called systems of cells, the best known of which is Aschoff's[7] *reticulo-endothelial system*. The term is not a good one, however, for, as indicated above, the cells of the endothelium proper are not phagocytic; although called endothelial cells, the phagocytic littoral cells (such as Kupffer cells) are similar to or identical with reticular cells. Perhaps a better designation is simply *macrophage system* or the broader term *lymphoid-macrophage system*,[8] which includes the lymphoid cells and the transitional polyblasts.

Local Defense.—The cellular response to an inflammatory stimulus[9] is characterized by an initial migration of polymorphonuclear leucocytes to the point of injury. These cells are not numerous and soon disappear when the inflammatory material is sterile, but when bacteria are present they continue to migrate from the blood vessels and actively phagocytose the invading microorganisms. The functions of this first line of defense, although important, are strictly limited, since the cells are short-lived and do not multiply *in situ* but must be continuously recruited from the blood stream.

[7] Aschoff: Ergebn. inn. Med. Kinderheilk., 1924, *26*:1.

[8] Taliaferro and Mulligan: Indian Medical Research Memoir No. 29, 1937.

[9] *Cf.* Menkin: *The Dynamics of Inflammation*. The Macmillan Company. New York 1940.

More important to local defense are the lymphoid cells of the blood and lymph, the lymphocytes and monocytes, which also migrate from the blood vessels but which, unlike the polymorphonuclear leucocytes, are long-lived and multiply in the tissues. These stem cells may be transformed to macrophages which, together with macrophages already present in the area, actively phagocytose and digest the invading microorganisms. When large bodies of foreign material are present, the macrophages may fuse to form foreign body giant cells; when microorganisms are indigestible, they may form giant cells around them such as the epithelioid cells of the tubercle. Their progressive development into fibroblasts supplies the active elements for regeneration and repair, the formation of scar tissue and the walling off of foreign bodies.

Certain other cells may take part in the late stages of the inflammatory process; the eosinophils, for example, may play a part in the detoxification of proteins and their disintegration products.

General Defense.—When a stimulant is distributed over a large part of the body the reaction is regarded as a general rather than local one; such distribution, however, usually means that the stimulant is in the blood stream and is combated by the cells of organs most closely associated with the blood, *i.e.*, the spleen, liver and bone marrow, and in some cases general reactions may be regarded as local ones in strategically placed organs.[10] The same types of cells are involved as in the local reaction, the polymorphonuclear leucocytes being mobilized first, with the macrophages playing the more important rôle. Endothelial cells, although in contact with blood-borne material, show very little phagocytic activity and the fibroblasts are rarely active.

The Cellular Response in Immunity.—The above reactions, which are observed to occur in the non-immune animal, are markedly accentuated in the immune animal, and there is an increase in the number of macrophages frequently designated as a hyperplasia of the reticulo-endothelial system. The cooperative rôle of the humoral antibodies is of considerable significance; an antigen is localized by agglutination if cellular or by precipitation if in solution, and in either case the material is made more readily phagocytable by opsonization. Bacteria injected into the blood stream, for example, are rapidly removed in the immune animal by the fixed macrophages of the liver and spleen, and staphylococci injected into the skin are localized and phagocytosed in great numbers.[11] When antigen and antibody meet in the tissues of an immune animal, there are not only localization and opsonization of the antigen, but also a much heightened inflammatory reaction including a speeding up of the cellular response.

The Origin of Antibodies.—Not only are the humoral antibodies and the cells of the macrophage system functionally interrelated as a part of the defense mechanism of the body, but there is good reason to believe, although no absolute proof, that the antibodies are formed by these cells. It will be clear that the antibodies, *i.e.*, globulin, are synthesized somewhere in the body and probably such synthesis is intracellular. That the

[10] *Cf.* Taliaferro: Jour. Parasit., 1934, *20:*149.
[11] *Cf.* Cannon: Physiol. Rev., 1940, *20:*89.

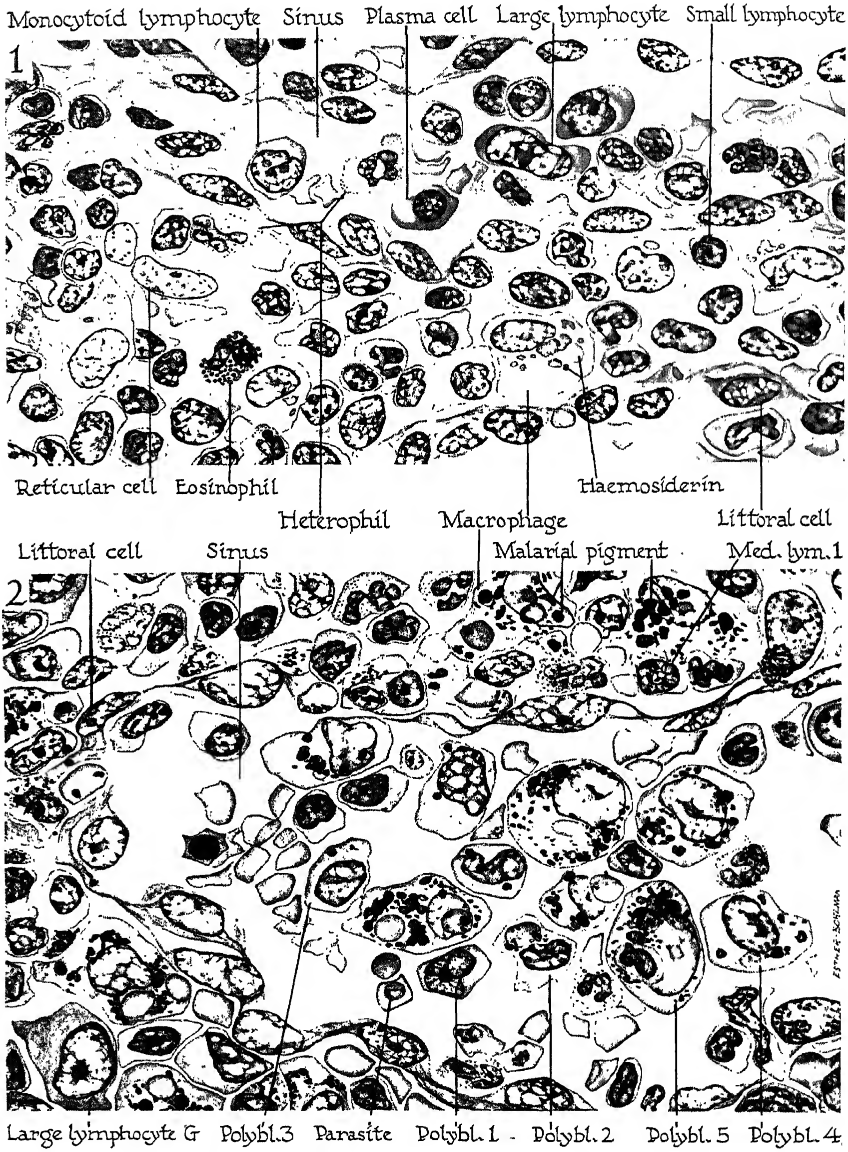


Fig. 35.—Cells of the macrophage system. Sections of monkey spleen showing a portion of a venous sinus and a Billroth cord in the red pulp. *1*, Normal monkey; note the typical structure of the non-granular leucocytes, the reticular cells with indeterminate cytoplasm, and the rounded macrophages within the cord. 2, Monkey killed ten days after infection with *Plasmodium cynomolgi;* note the large phagocytic cells absent from the normal spleen; the series of polyblasts labelled Polybl. 1–5 illustrate the progressive hypertrophy of the non-granular leucocytes into macrophages. Hematoxylin and eosin-azure II; × 1240 (Taliaferro and Mulligan).

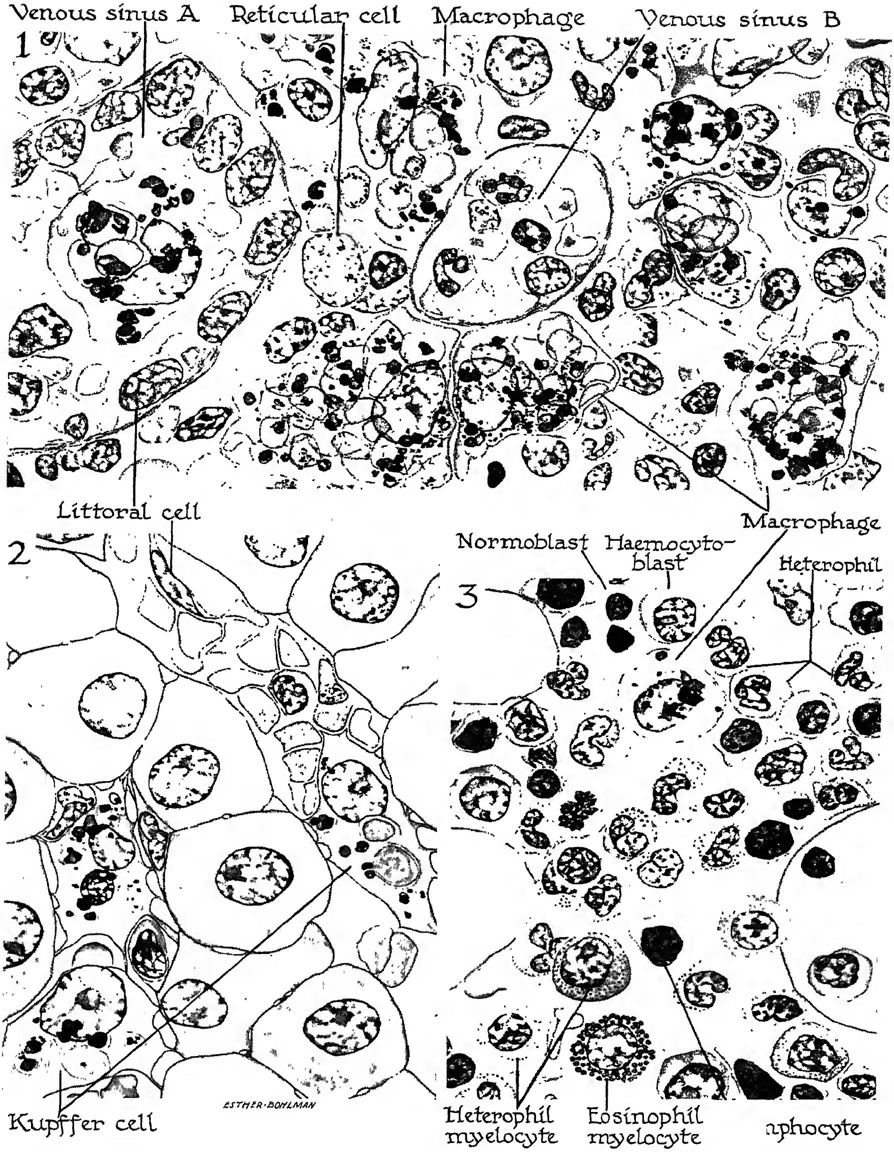


Fig. 36.—Cells of the macrophage system. Sections of spleen, liver and bone marrow from a monkey killed fifteen days after infection with *Plasmodium cynomolgi*. *1*, A Billroth cord and two venous sinuses from the spleen; note the phagocytic activity of the macrophages and smaller polyblasts. 2, Section from the liver; the Kupffer cells are phagocytic but here less active than the spleen macrophages. *3*, Section of bone marrow, showing a single, relatively small macrophage. Hematoxylin and eosin-azure II; × 1240 (Taliaferro and Mulligan).

cells of the lymphoid-macrophage system are more closely associated with antibody formation than other body cells is indicated by experiments in which the production of antibodies is interfered with by removal of tissues containing large numbers of these cells, such as the spleen, or by "blockade" of the macrophage system through the injection of india ink or similar substances which are phagocytosed to such an extent that the cells are fully occupied and unable to respond in a normal fashion to an antigenic stimulus.

Local Immunity.—The defense mechanisms functioning in the immune state have been assumed to be general ones and, for all practical purposes, equally effective throughout the body. It has been suggested, however, that these mechanisms are localized, or obviously accentuated, in certain tissues, not necessarily in tissues containing a large proportion

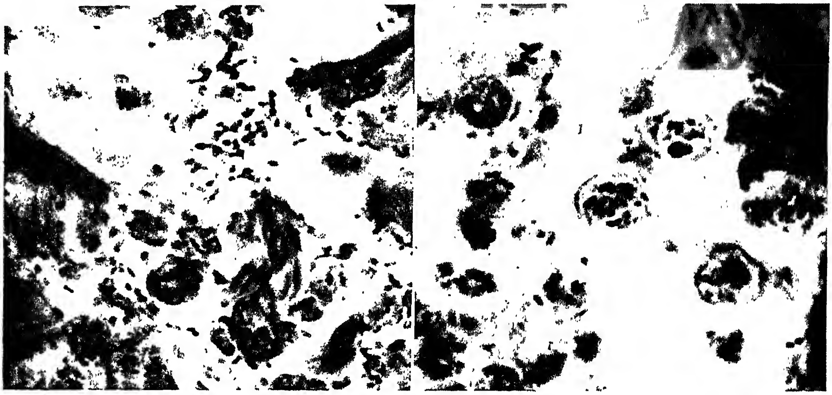


Fig. 37.—The cellular response in the immune animal. Rabbits injected subcutaneously with virulent pneumococci. Left, normal animal showing a typical spreading lesion with many extracellular pneumococci and minimal phagocytosis by polymorphonuclear leucocytes. Right, immune animal, showing marked phagocytosis with intracellular agglutination and few extracellular bacteria. Sections stained by Gram's method; × 1050 (Cannon).

of cells of the macrophage system, but in tissues such as the skin, the intestinal mucosa, the nasal mucosa, etc.

Although attributable in some degree to the nature of the local defense factors, such as *p*H and the like, the predilection of an invading microorganism for some particular part of the body such as the central nervous system might be regarded as indicative of a relative susceptibility of certain tissues and, conversely, a relative resistance on the part of others—in Ehrlich's terminology, the presence or absence of cell receptors. In a disease such as typhoid fever or erysipelas, for example, it should be necessary then that only the intestinal mucosa or the skin be immune, the other tissues being already resistant. This concept has been advocated particularly by Besredka.[12] The evidence upon which it has been based is generally regarded

[12] Besredka: *Local Immunization*. Williams & Wilkins Company. Baltimore. 1927.

as relatively weak, and local immunity in Besredka's sense probably does not exist.

There is, however, good reason to think that local immunity, not of a particular tissue no matter where it may be in the body such as the skin, but of a local area, does exist. It would appear to follow that the presence of antigenic material in a particular area would stimulate the production of antibody by the cells of that area and, in consequence, higher antibody titers would be found in the inoculated and immediately adjacent area than in the blood stream and other tissues. Such higher antibody titers have been demonstrated[13] in the nasal mucosa, for example, and it appears probable that, under circumstances in which antibody production takes place within a restricted area, that area may exhibit an accentuated immunity.

Natural Immunity.—As pointed out elsewhere (p. 187), the defenses of the normal animal against infection are of two general types. The first, characterized by non-specificity with respect to the infectious agent, has been called resistance and discussed earlier. The second type, showing a greater or lesser degree of specificity, is considered here as natural immunity.

It is the rule rather than the exception that pathogenic microorganisms are sufficiently closely adapted parasites that they are able to establish infection only in more or less closely related species of hosts. Thus it is perhaps not to be expected that the same parasite will be able to infect organisms as widely different as higher plants and mammals. It is of particular interest, therefore, that *Pseudomonas pyocyaneus*, a not uncommon pathogen of man and higher animals, has been found able to infect tobacco plants and may be identical with the plant pathogen *Phytomonas polycolor*.[14]

While in Ehrlich's terminology it might be said that the cellular organization of the plant lacks receptors for the human pathogen, there is evidence of a positive type of resistance to such infection. Antibiotic substances, similar to those produced by bacteria and fungi (p. 132), have been found in a number of higher plants. Thus, extracts of cabbage, turnip, onion and barberry plants inhibit the growth of *Bacterium coli* and *Bacillus subtilis*,[15] chlorophyll and related compounds have been found to inhibit the growth of the tubercle bacillus,[16] and substances which inhibit the growth of *Staphylococcus aureus* and *Bacterium coli* have been found in a large number of angiosperms.[17] None of the plants, however, is known to produce anything resembling antibodies.

Natural immunity is closely associated with the specific immune response, an immunity which is expressed in the form of the bactericidal and

[13] Cannon *et al.*: Proc. Soc. Exp. Biol. Med., 1931, *29*:517, 675; Hartley: Jour. Inf. Dis., 1940, *66*:44.

[14] Elrod and Braun: Jour. Bact., 1942, *44*:633.

[15] *Cf.* Sherman and Hodge: Jour. Bact., 1936, *31*:96; Dick: Arch. Surg., 1940, *41*:287; Fuller and Higgins: Food Research, 1940, *5*:503.

[16] Daly, Heller and Schneider: Proc. Soc. Exp. Biol. Med., 1939, *42*:74.

[17] Osborn: Brit. Jour. Exp. Path., 1943, *24*:227; see also Pratt *et al.;* Science, 1944, *99*:351; Tsuchiya *et al.*: Jour. Bact., 1944, *47*:422; Lucas and Lewis: Science, 1944, *100*: 597.

opsonic powers of normal blood, in the presence of the so-called normal agglutinins and the like. Such antibodies are generally present only in low titer, and the immunity associated with their presence in man is of a low grade but is possibly a significant part of that complex designated "resistance" in an earlier chapter. In many instances antibodies may be found under circumstances in which it is highly improbable that the animal has come in contact with the infectious agent; it has been noted, for example, that cattle sera will not infrequently neutralize the yellow fever virus and, more often, contain agglutinins for bacteria such as the typhoid and dysentery bacilli, the cholera vibrio, and certain of the rarer varieties of *Salmonella.* Although present in low titer, such normal agglutinins appear to be no different from the immune agglutinins in adsorbability, heat resistance and the like.[18] The normal macrophage response does not involve this specific element, for all kinds of intact particles are phagocytosed. The question of the origin of these antibodies is, then, of some significance and it may be asked whether or not specific antibodies may be formed in the absence of antigenic stimulation.

Natural Antibodies.—That the formation of some antibodies is genetically determined and takes place in the absence of antigenic stimulus is established in the case of the antibodies which determine the human blood groups. It is not unreasonable to suppose that in other instances the arrangement of polar forces on the surface of the normal globulin molecule might be such as to make possible to a greater or lesser extent the specific adsorption of antigenic substances. The probability that this will occur is, however, not great, and it is unlikely that such an explanation is generally valid.

Inapparent Infection.—A second possibility that accounts for the presence of normal antibodies is that of inapparent infection. A case in point is that of the appearance of diphtheria antitoxin in the blood in the absence of clinically recognizable infection; it is well known that the proportion of Schick-negative individuals increases in succeeding age groups. It is definitely established that the indicated formation of antitoxin is an immunity resulting from inapparent infection, *i.e.*, the carrying of virulent diphtheria bacilli in the throat, and the antigenic stimulus of the presence of small amounts of diphtheria toxin (p. 565). The agglutinins for Flexner dysentery bacilli and some of the common species of *Salmonella* associated with food poisoning not infrequently present in normal human sera to titers of 1:80 to 1:160[19] in all probability are a consequence of subclinical infections. It is probable that such a sequence of events occurs in a number of diseases and that the so-called normal antibodies, such as the neutralizing power of normal adult serum for poliomyelitis virus, measles virus and other infectious agents, are, in fact, immune antibodies. The manner in which a pseudoracial immunity may be produced through infection has been discussed elsewhere (p. 189).

Common Antigens.—As indicated above, however, there are many instances in which it is extremely unlikely that the animal whose serum agglu-

[18] Jordan: Jour. Inf. Dis., 1937, *61:*79.

[19] For example, see Schwabacher, Ross and Carruthers: Lancet, 1943, *i:*739.

tinates a given bacterium came in contact with it either as a mild infection or otherwise. It is difficult to conceive, for example, of the ordinary cow in the United States coming in contact with the cholera vibrio. In such cases it is not necessary to assume either that the observed antibodies arose *de novo* through some genetic or maturation mechanism or that there was contact with the particular microorganism. It is only necessary to assume that the animal has come in contact with an immunologically similar antigen. The likelihood that this will occur is clearly a function of the frequency with which immunological relationship or identity is shared by diverse organisms and the probability of contact with the unrelated form.

In addition to heterophile antigen, whose apparent random occurrence has been discussed elsewhere, immunologically similar antigens have been found to occur in a variety of seemingly unrelated organisms, and it is probable that many more have not as yet been discovered. For example, cross-reactions occur between Type II pneumococcus polysaccharide and Type B Friedländer's bacillus, certain species of yeasts, gum acacia and other vegetable gums, and some strains of *Bacterium coli* and *Bacterium aerogenes;* the polysaccharide of Type XIV pneumococcus is immunologically similar to the specific antigen of human blood group A; a constituent present in peptone is immunologically related to certain streptococcus antigens (group C); the capsule of the anthrax bacillus appears to be immunologically identical with that of *Bacillus mesentericus;* the plague bacillus and paratyphoid B are immunologically related. From these examples[20] it will be clear that immunologically similar antigens may be distributed in an apparently random fashion in unrelated organisms and it is probable that in some, if not many, instances in which antibodies may be demonstrated for some microorganism with which infection or contact is unlikely, the antibodies are immune rather than "normal," and a consequence of exposure to a similar antigen.

In keeping with this discussion it may be pointed out that there is a strong possibility that there is no such thing as a normal specific antibacterial immunity and that immunity in which a specific antibody may be demonstrated is one that arises as a consequence of exposure to the antigen. The incubation period following injection, for example, provides the opportunity for an immunological response that is difficult to rule out in many instances. In this connection it is of interest that attempts to establish a monoflora of *Bacillus subtilis*, an organism generally regarded as a harmless saprophyte, in bacteriologically sterile rats have resulted in a number of deaths from *B. subtilis* septicemia.[21]

Acquired Immunity.—Specific immunity against infection may be of two types. The one, *active immunity*, is due to the direct participation of the host and is gained at the expense of the organism acquiring it. This active immune state, manifested by the presence of antibodies, a heightened cellular reactivity, and a marked increase in resistance to the infection, arises as a consequence of the stimulus of antigen present in the tissues. The antigen may be inadvertently present, as in an attack of disease, or

[20] The literature is reviewed by Ingalls: Jour. Immunol., 1937, *33*:123.

[21] Personal communication from Dr. J. A. Reyniers.

it may be purposely introduced into the tissues in such a form that, while its antigenic qualities are little if at all impaired, disease is not produced. Active immunity may, then, be artificially produced by the parenteral inoculation[22] of appropriate antigenic material. In general terms, the methods by which active immunization may be accomplished are:

(1) The inoculation of living, fully virulent bacteria
  (*a*) rarely by a route favorable to infection but
  (*b*) occasionally by a route unfavorable to infection or
  (*c*) in conjunction with protective antiserum;
(2) the inoculation of attenuated bacteria of greatly reduced virulence for the host;
(3) the inoculation of bacteria killed by
  (*a*) heat or
  (*b*) antiseptics;
(4) the inoculation of bacterial products
  (*a*) secreted during life or
  (*b*) liberated by the autolysis of dead cells;
(5) the inoculation of bacteria unrelated to the production of the specific infection.

These may be briefly illustrated.

(1) Immunity produced by the introduction of living, virulent bacteria is practically identical with the immunity that results from an attack of disease after natural exposure. In experimental work the varying facility with which this mode of immunization can be effected is in part dependent upon the susceptibility of the organism to the particular parasite, and a highly susceptible animal can be immunized in this way only with great difficulty or not at all. The successful use of living cultures involves the administration of small non-fatal doses which are increased as immunity develops.

The relative insusceptibility to infection by some particular route may be taken advantage of, as in Ferran's method, now superseded, for protective vaccination against Asiatic cholera; natural infection occurs via the alimentary tract and subcutaneous injection of the virulent vibrios is followed by a local inflammation but not by a general infection with serious consequences.

The simultaneous administration of virulent microorganisms and protective antiserum provides preformed antibody for combating the invader while allowing the immune reaction of the host to develop. This method is seldom used for the immunization of human beings but is common in some other instances; hogs may be immunized against hog cholera, for example, by simultaneous injection of virus-containing blood and antiserum.

(2) As indicated elsewhere, the virulence of a bacterial culture may be greatly diminished in a variety of ways, such as cultivation under unfavorable environmental conditions and by passage through animal species other than that of the host in question. Perhaps the most familiar examples of the use of such attenuated material are those of smallpox vaccination and inoculation against rabies. In the first instance the passage of smallpox

[22] Oral administration of antigenic material is efficacious only in so far as unmodified antigen is absorbed through the intestinal wall and, compared with parenteral inoculation, is an uncertain and inefficient method.

virus in the cow greatly reduces its virulence for man but inoculation with the altered virus of cowpox protects against smallpox infection, and in the second rabies virus from dogs is "fixed," in Pasteur's terminology, or attenuated by serial passage in rabbits. This method is particularly useful in immunization against the filterable virus diseases, since cultures are not available and "killing" destroys the antigenic properties of these agents. (See, however, p. 798.)

(3) Suspensions in physiological salt solution of bacteria killed by heating to 55 to 60° C. for thirty minutes or by treatment with formaldehyde or phenol are widely and successfully used in man against typhoid and paratyphoid fevers and against a variety of microorganisms in the laboratory. Such vaccines are, in general, most useful in infections in which the microorganism does not produce a soluble toxin but which contains an endotoxin. This method has the obvious advantage of avoiding all danger of infection while at the same time introducing into the body the substances most intimately connected with the bacterial cell and its activities.

(4) The use of products of the bacterial cell for immunization purposes finds widest application in the case of those organisms which produce soluble toxins. As pointed out above, a solid immunity against diphtheria may be secured by stimulating the production of antitoxin. It is possible to build up such an immunity by using extremely small amounts of unmodified toxin in the early injections with gradual increases as immunity develops. In general, however, it is much more satisfactory to use neutralized or detoxified toxin. In the first instance a neutral mixture of diphtheria toxin and antitoxin (TAT) is frequently used with satisfactory results; the complex breaks down slowly in the body, liberating free toxin which acts as the antigen. Toxoid prepared by treatment with formalin which has lost its toxicity but retained its antigenicity is generally used at the present time in a partially purified form precipitated by alum. The alum-precipitated toxoid is of some advantage since the toxin-alum complex breaks down slowly in the tissues, liberating toxoid slowly to provide a prolonged antigenic stimulus.

It is difficult to distinguish between bacterial products secreted during life and the products of autolysis; it has been pointed out previously, for example, that usually the soluble toxin titer of a culture rises, not coincident with the phase of active growth, but after cell multiplication slows down and breakdown of the formed cells begins. Studies have been made, however, on the antigenic qualities of autolyzed cultures and of cell extracts, but such antigens are not of great practical value since they are contained, for the most part; in suspensions of killed cells.

(5) Some degree of immunity toward specific infections may be developed by the use of certain kinds of bacteria or bacterial products entirely foreign to the infection in question. In this category, for example, is the undoubted protection conferred against anthrax by the injection of *Bacterium prodigiosum* or *Ps. pyocyanea* or their products. A number of instances of this sort have been described in connection with studies on common antigens and need not be considered further here.

Irrespective of the antigen employed, the animal body requires a period of time to respond with antibody production and for a high degree of immunity successive antigenic stimuli are required. Early injections may consist of killed bacteria, followed by the inoculation of living, attenuated or virulent cells. The process of forcing a very high degree of immunity upon an experimental animal is often termed *hyperimmunization.* If the antigen is injected in such a form that it is slowly absorbed, thus providing a prolonged stimulus, repeated injections may not be necessary. A single injection of alum-precipitated diphtheria toxoid, for example, may give about 80 per cent Schick negatives. Attempts have been made to use *lipo-vaccines*, suspensions of bacteria in oil, to promote such slow absorption, but these are generally unsatisfactory because they are difficult to sterilize.

An animal may be simultaneously immunized against more than one antigen with no significant effect on the immune response. Typhoid vaccine, for example, commonly contains typhoid, paratyphoid A and paratyphoid B bacilli (TAB), and other so-called polyvalent vaccines containing a variety of microorganisms are effective immunizing agents.

The immunity produced is variable to some degree and dependent upon the efficacy of the antigen used. Some kinds of bacteria are "good antigens," such as the typhoid bacillus, while others, such as the gonococcus, are "poor antigens" and no effective immunity may be produced. The reason for this is not known. When an active immunity is produced, however, it is generally of long duration and effective over a period of years.

The immune response, as indicated by antibody titer, generally is apparent by the second of a series of injections and the titer increases with succeeding injections until an upper limit is reached. With cessation of inoculations the antibody titer slowly declines and within a few weeks has reached a very low level. Subsequent injection brings about an immediate antibody response, much more rapid and pronounced than that of the initial immunization. It is of some interest that this secondary response may be brought about, although to a lesser degree, by the injection of a heterologous antigen, a phenomenon that has been termed the *anamnestic reaction;* this stimulation to fresh production of one antibody when a new antigen is injected has been an important obstacle to theories of antibody production such as retention of antigen in the cells, etc. It may be noted here that the injection of any antigenic substance stimulates the mobilization of the defense mechanisms of the body, a phenomenon which is made use of in *non-specific protein therapy;* the injection of sterile milk, for example, may markedly stimulate the body to combat invading microorganisms. It will be clear that, under the circumstances, any interpretation of the beneficial effect of vaccine therapy of infectious diseases must be made with caution.

Passive Immunity.—In contrast to active immunity, passive immunity involves no active generation of protective substances by the immunized animal. The latter is simply the recipient of antibodies formed in the body of another animal and transferred to the individual to be protected. Such passive transfer occurs in nature from mother to offspring, *in utero* via the placental circulation in man, apes, and rodents in which there is only one

layer of cells between the maternal and fetal circulatory systems, and after birth via the colostrum in ruminants in which there are four layers of cells between the two circulatory systems.[23]

Artificial passive immunization is brought about by the injection of immune sera such as the antitoxic sera of horses immunized against diphtheria toxin, tetanus toxin and similar antigens, or of convalescent sera taken from recovered human cases of the disease in question. Passive immunization is most effective with antitoxic sera; with exceptions such as virus neutralizing sera, antipneumococcus sera and the like, antibacterial sera are generally not highly effective either prophylactically or therapeutically.

Unlike active immunity, passive immunity is not of long duration, generally not more than two or three weeks. Repeated injections of tetanus antitoxin, for example, must be made at weekly intervals as long as the danger of infection remains. Horse serum globulin acts as a foreign protein in the human body and tends to be eliminated as such. Passive immunization with homologous serum is possibly of somewhat longer duration.

Hypersensitivity.—The response of the animal body to the presence of antigen in the tissues is one that is of obvious advantage, particularly when the antigen is a toxin or the cell substance of an invading microorganism. That the immunological response does not always have desirable consequences was early apparent when it was observed that an otherwise harmless antigen, such as egg albumin, may prove to be highly toxic on second injection. The experimental animal reacting in this way is said to be sensitized, or rendered hypersensitive, to the antigen by the first injection. Differences in the clinical manifestations of the reaction of the organism to the antigen have resulted in a series of "kinds" of hypersensitivity which may be considered briefly.

Anaphylaxis.—The term anaphylaxis (against protection) was used by Richet[24] to denote the hypersensitivity acquired by laboratory animals in response to the injection of antigen, and the syndrome following subsequent injection is designated anaphylactic shock. Zinsser[25] has described the anaphylactic reaction in the guinea pig as follows: "If a sensitized guinea pig receives a second injection of an antigen after a suitable incubation time, a characteristic train of symptoms ensues. There is usually a short preliminary period, lasting either a fraction of a minute or several minutes according to the violence of the reaction and the mode of administration. At the end of this time the animal grows restless and uneasy, and often rubs its nose with its forepaws. It may sneeze and cough. At the same time increased respiration is noticeable and the fur appears ruffled. In light cases the animal may remain in this condition, with further difficulty of respiration, discharges of urine and feces; then gradual recovery sets in, with complete return to normal in several hours. In more severe cases these

[23] See the review in the Lancet, 1941, *i:*44.

[24] Portier and Richet: Compt. Rend. Soc. Biol., 1902, *54:*170; Richet: Ann. Inst. Pasteur, 1907, *21:*497.

[25] Zinsser, Enders and Fothergill: *Immunity Principles and Application in Medicine and Public Health.* The Macmillan Company. New York. 5th ed., 1939. p. 349.

preliminary stages are rapidly followed by weakness. The animals fall to the side, the legs and trunk muscles twitch, and the respiration becomes slow and shallow. The dyspnea is inspiratory. The excursions of the lung grow shallower in spite of strong inspiratory efforts, the volume of the thorax and lung remaining in the expanded condition. At this stage the animal may arise and attempt to run. More often, however, general convulsions set in, and in these the animals usually die. The heart continues to beat for a considerable time after breathing has stopped." On autopsy the lungs are distended because of the imprisonment of the air in the alveoli by the contracted musculature of the small bronchioles; small hemorrhagic areas are found on the serous surfaces of the lung and in the brain, stomach, heart, cecum and spleen, and there are local fatty changes in the capillary endothelium.

Both rabbits and dogs may be sensitized and suffer from acute anaphylactic shock with, however, significant differences from the guinea pig. In the rabbit pulmonary dilatation is lacking but there is extreme dilatation of the right side of the heart and death is a consequence, not of suffocation, but of spasmodic contraction of the branches of the pulmonary artery and acute heart failure. In the dog, on the other hand, the outstanding postmortem feature is the congestion and distention of the liver.

There are, however, certain basic features of the anaphylactic reaction, such as a reduction in complement and fibrinogen in the blood, leucopenia, capillary dilatation and general irritability of the smooth muscle. The last may be demonstrated *in vitro* as the contraction of a strip of uterus from a sensitized guinea pig in response to the presence of antigen. Anaphylactic shock appears to be primarily a cellular reaction in which the primary point of attack is the cells of the macrophage system; the difference in symptoms in different animal species arises as a consequence of the distribution of reactive cells and their relative ability to anchor antigen.

Like the other immune reactions, hypersensitivity is highly specific, is passively transferable, and does not appear until after a suitable period of incubation. The last is somewhat variable depending upon the amount and kind of antigen injected; in the case of sensitization of the guinea pig to horse serum it is ten to twelve days. If a second injection is given one to three days after the first, the appearance of hypersensitivity is delayed but if eight to ten days intervene, hypersensitivity and susceptibility to anaphylactic shock do not appear, a condition which has been termed *anti-anaphylaxis*. When developed, however, the hypersensitive state persists for long periods in experimental animals, probably for life. Sensitization may be effected by very small quantities of antigen; in one instance 0.0000001 cc. of horse serum was sufficient to render a guinea pig sensitive.[26] Wells,[27] using pure protein, has shown that the minimum sensitizing dose of crystallized egg albumin is 0.00000005 gm. and the minimum fatal sensitizing dose 0.000001 gm., the fatal reacting dose being intravenously

[26] Sensitization to horse serum was thoroughly studied by Rosenau and Anderson: Bulls. 29, 30, 36, Hyg. Lab. Mar. Hosp. Service; Jour. Inf. Dis., 1908, 5:85.

[27] *Cf.* Wells: *Chemical Aspects of Immunity*. Chemical Catalog Co. New York. 2nd ed., 1929.

0.00005 gm., and intraperitoneally 0.0005 gm. Anaphylactic shock may also be brought about by the injection of haptenes although these partial antigens will not sensitize. Animals that survive the second dose of antigen are *desensitized* or temporarily refractory, but sensitivity returns within a variable time.

The Schultz-Dale Reaction.—The irritability of the smooth muscle characteristic of anaphylactic shock may be demonstrated *in vitro* with an isolated strip of intestine or the uterus of a sensitized guinea pig. The piece of tissue is suspended in oxygenated Ringer's solution and the antigen added to the bath. The tissue responds with a vigorous contraction, very similar to that produced by histamine, which may be recorded with a kymograph. No reaction is produced by a second addition of antigen for the tissue is desensitized.

The Arthus Phenomenon—Local Anaphylaxis.—It was noted by Arthus[28] that in a series of subcutaneous injections of horse serum into the rabbit the earlier injections were without local effect but as the animal became immune the site of inoculation became swollen and edematous, at first transiently, but with succeeding injections the response took the form of a firm indurated area with local necrosis. This response was not confined to the neighborhood of earlier injections but could be elicited at any site. The phenomenon is demonstrable to only a limited extent in the guinea pig and not at all in other experimental animals but does occur in man. Such, for example, are the indurated areas which are apt to occur at the site of inoculation after the sixth or seventh injection of rabies prophylactic. The phenomenon appears to be dependent upon union within the tissues of circulating precipitin and its specific antigen.[29]

The Nature of the Anaphylactic Reaction.—It will be clear that the phenomenon of anaphylaxis is essentially an immunological one. It was pointed out earlier that the union of antigen and antibody in the tissues produces a marked inflammatory reaction. There is reason to believe that this is the explanation of anaphylaxis, the antigen-antibody (probably precipitin) union possibly taking place within the cells and the inflammatory reaction of the macrophage system exceeding safe limits. In the immune animal there is presumably sufficient circulating antibody to combine with and immobilize the antigen before it can reach the cells.

The question of why the union of antigen and antibody should have the observed consequences is another matter. Two theories have been proposed, one of which, the anaphylatoxin theory, assumes that the antigen is partially digested, perhaps through the agency of serum complement or by proteolytic enzymes, with the liberation of toxic substances (see p. 175). Symptoms similar to those of anaphylactic shock, the so-called *anaphylactoid reactions*, may be produced by the injection of peptone or larger products of protein cleavage and by histamine. The immediate response of isolated smooth muscle, however, throws doubt on this hypothesis. The second theory postulates a disturbance of the colloidal equilibrium in the blood stream or within the cell as a consequence of the antigen-antibody

[28] Arthus: Compt. Rend. Soc. Biol., 1903, *55*:817.
[29] Cannon and Marshall: Jour. Immunol., 1941, *40*:127.

union. In this connection it is of interest that blood serum shaken with kaolin or other adsorbing agents is found to be toxic on reinjection. There is a good deal of evidence which strongly suggests that the liberation of physiologically active substances, including histamine, heparin and possibly choline, occurs in anaphylactic shock. The symptoms can be explained on the basis of an autointoxication by such substances, normally present in the tissue cells and liberated by some change in permeability brought about by the antigen-antibody reaction.[30] While the injection of histaminase will not protect against anaphylactic shock, it has been reported[31] that immunization with artificial histamine-protein antigen will produce a significant degree of protection.

Hypersensitivity in Man.—The phenomenon of anaphylaxis, although occurring in nature to a limited extent, is essentially a laboratory phenomenon. Hypersensitivity may be manifested in a variety of ways, however, and, from the anthropocentric point of view, its manifestations in man are the most important. Human hypersensitivity has been variously termed *allergy* (the term was originally introduced by von Pirquet as a designation for hypersensitivity in general rather than any of its particular manifestations), *idiosyncrasy* and *atopy*. In general it may be said that in man there is little tendency to generalized shock; typical anaphylactic shock is occasionally observed, but, as in dogs and monkeys, there is a tendency to localization of manifestations particularly in the respiratory tract, the gastro-intestinal tract and the skin.

Serum Sickness.—The administration of antitoxic or antibacterial serum produces, in a certain proportion of people, a characteristic syndrome termed serum sickness. The symptoms include urticaria, fever, glandular swelling, edema and pains in the joints, in combinations that are variable from one individual to another. The reaction may occur after initial as well as subsequent injections and is attributable to the foreign products, usually horse serum, rather than to the antibody content of the serum.

Allergy.—Also included under the head of hypersensitivity is a group of reactions which have been designated the allergies. Such, for example, are the sensitivities to the pollens, horse dander, rabbit fur, cat fur and the like in which the reaction may be localized predominantly in the upper respiratory tract with the familiar symptoms popularly designated as hay fever. Another group of reactions in this category are those of individuals hypersensitive to certain food substances such as strawberries, eggs and milk, in which the manifestations tend to be localized in the gastro-intestinal tract but which may include urticaria and other symptoms. The drug idiosyncrasies are similar hypersensitive phenomena. This type of hypersensitivity is passively transferable. If a local area of the skin of a normal individual is infiltrated with serum from a hypersensitive person, that area will give a typical hypersensitive reaction to intradermal inoculation with the homologous antigen. This phenomenon is known as the *Prausnitz-Küstner reaction.*

It will be clear that the antigens toward which hypersensitivity is mani-

[30] The evidence for this is reviewed by Dragstedt: Physiol. Rev., 1941, *21*:563.
[31] Fell, Rodney and Marshall: Jour. Immunol., 1943, *47*:237.

fested are in some instances protein and in others nonprotein. The existence of such non-protein "antigens," together with the fact that hypersensitivity may exist without history of previous contact with a sensitizing dose of antigen, has led a number of workers[32] to assume that these constitute a type of hypersensitivity sufficiently different to require the new name *atopy*. The passive transfer of the types of sensitivity falling into this category is presumed to be due to the presence, not of the usual antibodies, but of similar substances designated as *reagins*.

The Relationship of the Various Forms of Hypersensitivity.—Although it may assume various clinical forms in man, it is probable that the various forms of hypersensitivity are substantially identical. There has been no conclusive evidence, for example, of any essential difference between the anaphylactic antibodies and the atopic reagins, and the apparent absence of antibody means only that antibody has not been detected by the methods utilized. Nor is the differentiation of the various forms of hypersensitivity in their basic features of practical value—"*entia non multiplicanda praeter necessitatem.*"

Apparent differences may be explained without great difficulty. The fact that some of the antigens are non-protein is not of great significance, for anaphylactic shock may be elicited by haptenes and in any case the combination of haptene, particularly when it contains reactive groups, with body protein to form complete antigen is a possibility that is difficult to eliminate. A negative history of previous exposure to a sensitizing dose, if proved, and this is probably a practical impossibility, loses some significance in the light of present information concerning the distribution of common antigens. Clinical differences may be explained by the mode of contact with the antigen; ragweed pollen suspended in the air, for example, will obviously come in contact with the upper respiratory tract and affect reactive cells there rather than in other parts of the body. It is highly probable, therefore, that all types of hypersensitivity are basically identical.

The Inheritance of Hypersensitivity.—It has long been suspected that hypersensitivity is associated with some genetically determined factor or factors, *i.e.*, that it tends to run in families. Proof of this is beset with the difficulties encountered in any study of human inheritance but studies[32] have given definite indication that such is the case. It is not the hypersensitive state itself that is inherited but rather a tendency toward ready development of hypersensitivity. Direct experimental evidence of hereditary predisposition to sensitization in the guinea pig has been reported by Chase[33] and by Jacobs, Kelley and Sommers.[34] Hypersensitivity may, of course, be passively transferred from mother to offspring. The inheritance of resistance to disease (p. 187) is possibly another matter.

Bacterial Allergy.—The cell substance of bacteria is, of course, antigenic and may act as a sensitizing agent. Anaphylaxis, for example, may be induced against bacterial protein. Sensitization similarly occurs in dis-

[32] *Cf.* Coca in Jordan and Falk: *Newer Knowledge of Bacteriology and Immunology.* University of Chicago Press. Chicago. 1928.
[33] Chase: Jour. Exp. Med., 1941, *73*:711.
[34] Jacobs, Kelley and Sommers: Proc. Soc. Exp. Biol. Med., 1941, *48*:639.

ease; perhaps the best known example is the hypersensitivity to the cell substance of the tubercle bacillus in tuberculosis (p. 581). The intradermal injection of a small quantity of antigen results in an inflammatory reaction in the sensitized person or animal. Similar skin reactions may be elicited in glanders, brucellosis and the like by injection of the specific bacterial cell substance, and these reactions are frequently of diagnostic value. These tests for hypersensitivity are not to be confused with skin tests such as the Schick test in which a neutralization of toxin is involved. The question of the relation of hypersensitivity to immunity to disease has been of some interest. It has been pointed out by Rich[35] that while in some instances hypersensitivity does not exert any appreciable effect on the response to infection, in others it is decidedly harmful to the host, and has not as yet been shown to be of marked benefit in any infection, though the last may be regarded as still an open question.

Hypersensitivity and Immunity.—The paradoxical nature of an immune response which can result in either a specific protection against pathogenic bacteria and their products or an equally specific and marked susceptibility to an antigenic substance is not too difficult to reconcile. Although seemingly resting upon scme factual basis, the dual nature of the immune response is more apparent than real for it rests upon a question of emphasis. The soluble bacterial toxins, for example, are potent poisons whose pharmacological activity is nullified by the formation of antitoxin, but primarily the same mechanism converts an innocuous antigen, such as egg albumin, which can be injected in relatively large quantities, to a specific poison for the sensitized animal. The protective aspect is emphasized in the first instance while the acquired toxicity appears more important in the second. The matter of emphasis is illustrated in an analogy drawn by an eminent worker in the field[36] who has pointed out that the warmth, light and other pleasing aspects of a grate fire are also present when the house burns down; both involve precisely the same mechanism yet the emphasis upon the consequences of the combustion is entirely different in the two cases.

[35] Rich: Physiol. Rev., 1941, *21*:70.
[36] Bronfenbrenner: Amer. Rev. Tuberc., 1937, *36*:293.

CHAPTER 14

THE STAPHYLOCOCCI

Family: *Micrococcaceae*. Genus: *Staphylococcus*. Usually parasitic. Cells occur singly, in pairs, short chains and irregular clusters. Gram-positive. Good growth on nutrient agar, white, orange or yellow in color. Dextrose, maltose, sucrose and often lactose fermented with formation of moderate amount of acid. Gelatin may be actively liquefied. Nitrate reduction variable. Some strains hemolytic. Type species: *Staphylococcus aureus*.

The bacteria most commonly found in boils, abscesses, carbuncles and similar suppurative processes in man belong to the group of staphylococci. The presence of staphylococci in pus was first shown by Pasteur[1] and later by Ogston.[2] Micrococci were obtained in pure culture by Becker[3] in 1883, but their causal relation to the suppuration of wounds and to osteomyelitis was first brought out by the work of Rosenbach[4] in 1884.

Morphology and Staining.—The spherical cells are generally aggregated in loose, irregular masses which have been likened to clusters of grapes, and have given the generic name to this organism. In a preparation made directly from pus or from a pure culture, not only irregular clusters of cells can be observed but also tetrads, pairs and short chains of cells. It is, then, often difficult to determine from stained preparations whether only true staphylococci are present or whether there is an admixture of streptococci or other forms. The irregular clusters are most consistently observed in preparations from cultures on agar media, preparations from broth cultures showing, as a rule, greater dispersion of the cells.

The staphylococci do not form spores, and motile varieties are very rarely observed. The ordinary aniline dyes stain the cells readily, and the great majority of staphylococci are gram-positive. The dimensions of the individual cocci vary within narrow limits, the diameter of the cells ranging between 0.7 and 0.9 μ.

The growth on agar media is abundant, opaque and glistening, and the individual colonies are circular with entire edges. Pigment may be formed, a golden yellow in the case of *Staphylococcus aureus* and lemon yellow in the case of *Staphylococcus citreus*, but it is absent and the growth appears white in the case of *Staphylococcus albus* and other less common species.

Physiology.—The optimum temperature is 28° to 37° C., the latter in the case of those species found in association with man and lower animals; but growth can also take place at temperatures as high as 42° C. and as low as 8° to 9° C. The best known species, sometimes designated the aerobic staphylococci, grow best in the presence of oxygen and to some extent

[1] Pasteur: Bull. de l'Acad. de Med., 1880, *9*:447.
[2] Ogston: Brit. Med. Jour., 1881, *1*:369.
[3] Becker: Deut. med. Wchnschr., 1883, *9*:665.
[4] Rosenbach: *Mikroorganismen bei d. Wundinfektionskrankheiten.* Wiesbaden. 1884.

under anaerobic conditions; certain other species are, however, strict anaerobes. The staphylococci in general are not fastidious in their nutritive requirements and grow readily upon the ordinary nutrient media. Investigation of *Staphylococcus aureus* has shown that, although frequently requiring amino acids at first, this species may in time be grown with an ammonium salt as a sole source of nitrogen.[5] Both nicotinic acid and thiamin (aneurin, or vitamin B_1), together with an organic source of sulfur are, however, required,[6] and uracil[7] must be supplied for anaerobic growth. Growth in such synthetic solutions is greatly improved by the addition of biotin (bios II_B, vitamin H), and this substance may also be required by this microorganism.[8]

In the fermentation of glucose, lactic acid is the predominating end product (77 to 91 per cent), together with small quantities of ethyl alcohol and carbon dioxide.[9] The golden yellow pigment which distinguishes the *aureus* variety from other staphylococci is formed most abundantly upon

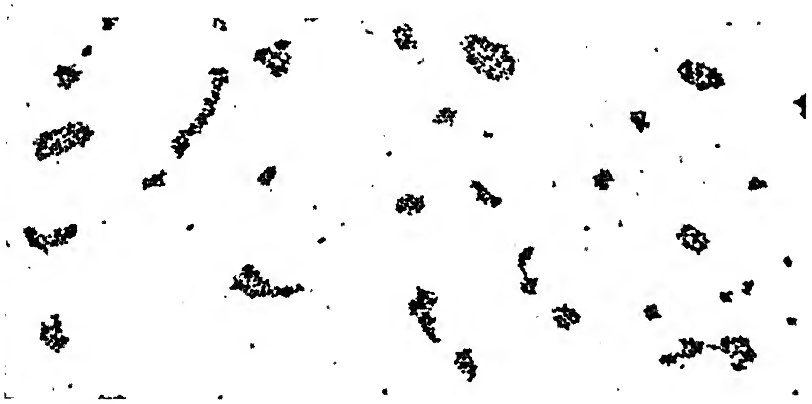


Fig. 38.—*Staphylococcus aureus* from pure culture. Note the characteristic clusters of the cocci. Fuchsin; × 1050.

carbohydrate media or blood serum in the presence of free oxygen. The pigments produced by the chromogenic staphylococci are probably lipochromes. A specific gelatin-liquefying enzyme or gelatinase is formed in the majority of gelatin and broth cultures and has been separated from the cultures by filtration. Other enzymes, such as rennin and maltase, are produced under suitable conditions. Dextrose, lactose, sucrose, maltose, glycerol and mannitol are usually fermented with acid but no gas, while raffinose, salicin and inulin are not attacked nor is starch hydrolyzed. Indol is not produced and nitrate reduction is variable. Milk inocu-

[5] Gladstone: Brit. Jour. Exp. Path., 1937, *18*:322.
[6] Knight: Biochem. Jour., 1937, *31*:966.
[7] Richardson: Biochem. Jour., 1936, *30*:2184.
[8] Kögl and van Wagtendonk: Rec. trav. chim., 1938, *57*:747; Porter and Pelczar: Science, 1940, *91*:576.
[9] Friedemann: Jour. Biol. Chem., 1939, *130*:61.

lated with staphylococci is usually coagulated by the acid resulting from fermentation, but the precipitated casein remains, as a rule, undissolved.

The thermal death point is not constant, different strains appearing to vary greatly in their resistance, some succumbing only after thirty minutes' exposure to a temperature of 80° C., while others are destroyed at a much lower temperature. Considerable resistance is displayed toward drying, experiments showing a retention of vitality for many days and even months in cultures dried upon silk threads and desiccated over calcium chloride. Toward the chemical substances ordinarily used as disinfectants the staphylococci also exhibit more than the average resistance. They are, in general, among the hardiest of the non-spore-forming bacteria.

Toxins.[10]—A variety of toxic substances are produced by staphylococci, including hemolysins, leucocidin, coagulase, fibrinolysin, spreading factor (Duran-Reynals factor), skin-necrotizing substance, a lethal factor and enterotoxin.

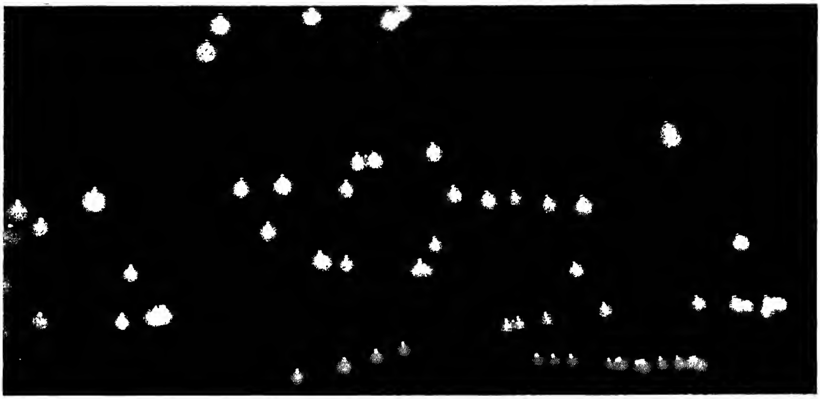


Fig. 39.—Colonies of *Staphylococcus aureus* on nutrient agar. Twenty-four-hour culture; × 3.

Hemolysins.—The pyogenic staphylococci are almost invariably β-hemolytic on blood plates and produce filterable hemolysins in broth culture, while the saprophytic forms are less frequently hemolytic. The filterable hemolysins (staphylolysins) are of two types, one lysing red cells upon incubation (α-lysin) and the other (β-lysin) only after holding in the icebox following preliminary incubation—the so-called "hot-cold" lysis (p. 178).

Other Toxins.—The ability of many pyogenic staphylococci to coagulate citrated plasma, lyse fibrin clots, kill leucocytes and increase the permeability of the skin may be shown by appropriate techniques. Sterile filtrates from broth cultures produce necrosis upon intradermal injection, the so-called skin-necrotizing factor, and almost immediate death when injected intravenously into rabbits. Whether these and other effects are

[10] *Cf.* Blair: Bact. Rev., 1937, *3*:97.

due to the activity of separate substances or various manifestations of the activity of a single substance is uncertain. It is definitely established that more than one hemolysin is produced and that the enterotoxic substance, effective *per os* and of considerable food-poisoning significance (p. 242), is not identical with the other activities. More than one toxic substance is produced, then, but probably some of these produce more than one effect. In general, toxin production is a property of the pathogenic staphylococci, usually of the *aureus* variety.

Variation.—Like other bacteria, the staphylococci dissociate, and both rough and G colonial types have been described.[11] These have not been thoroughly studied, however, and are not well known.

Classification.—The genus *Staphylococcus* is one of four which make up the family *Micrococcaceae.* The staphylococci are, in turn, roughly divisible into two types, the one designated as aerobic, though the bacteria are in fact facultative anaerobes, and the other which is strictly anaerobic. The species comprising the former group, which is by far the better known, are the pigmented forms *Staphylococcus aureus*, which is golden yellow and generally pathogenic, and *Staphylococcus citreus*, which is lemon yellow and a saprophyte, together with the non-pigmented *Staphylococcus albus*, *Staphylococcus epidermis*, *Staphylococcus muscae* and *Staphylococcus pharyngis*. The non-pigmented forms are saprophytic or only feebly pathogenic and are commonly not distinguished but lumped under the name of *albus*. There are three species of strict anaerobes, *Staphylococcus aerogenes*, *Staphylococcus asaccharolyticus* and *Staphylococcus anaerobius*. These anaerobic species are inhabitants of the body cavities, and the first is occasionally the cause of puerperal fever.

Physiological Differentiation.—The validity of these species is, however, open to serious question. The differentiation of the better known aerobic species on the basis of pigment production is of doubtful value, for this characteristic is variable. Recently isolated pyogenic cocci forming a rich golden pigment lose this property on continued cultivation on artificial media and become identical with the *albus* variety. No biochemical tests serve to sharply differentiate these bacteria from one another. The strains that produce white colonies are, as a rule, less active in gelatin liquefaction and fermentative power, and hence have been sometimes regarded as weakened relatives of the biochemically more vigorous golden pigmented types. The *albus* strains are also as a rule only feebly pathogenic. Dextrose, maltose and glycerol are fermented by nearly all *aureus* strains and by a consistently lower proportion of *albus* strains, lactose and mannitol by about four-fifths of the *aureus* and by about one-third (mannitol) to two-thirds (lactose) of the *albus* strains.[12] In general, the types most commonly isolated from air, dust, and other sources outside of the human body are white staphylococci, while those found associated with pathologic conditions are *aureus* strains. The general opinion is that the staphylococci form a closely graded series from the pigmented, hemolytic, gelatin-lique-

[11] Hoffstadt and Youmans: Jour. Inf. Dis., 1932, *51*:216; Bigger, Boland and O'Meara: Jour. Path. Bact., 1927, *30*:261; Swingle: Jour. Bact., 1935, *29*:467.

[12] Dudgeon and Simpson: Jour. Hyg., 1927, *27*:160.

fying, pathogenic, actively fermenting strains to those that are unpigmented, feebly pathogenic and less actively hemolytic, liquefying and fermenting.

Immunological Differentiation.—Differentiation of the staphylococci on the basis of agglutination reactions has as yet been unsatisfactory. In recent years, however, it has been shown[13] that two immunological types may be differentiated by precipitation tests with specific carbohydrates extracted from the microorganisms. One of these types, designated type A, is composed for the most part of strains isolated from pathogenic sources, while the other, type B, comprises strains of non-pathogenic origin. The majority of type A strains ferment mannitol, while those of type B do not.

The Coagulase Test.—The ability of staphylococcus strains to coagulate plasma appears to be associated with pathogenicity in that the majority of strains isolated from pathologic processes are coagulase-positive while the saprophytic strains are usually coagulase-negative. It has been suggested that the staphylococci be divided into "pathogenic" and "non-pathogenic" types on this basis,[14] and in recent years the coagulase test has been generally used.[15] It has been found also that coagulase-positive strains clump rapidly when mixed with fresh human plasma; the CO_2-soluble fraction of fibrinogen seems to be the most important part of the plasma in effecting immediate clumping.[16] The slide test[17] for clumping, carried out by mixing fresh, undiluted human plasma with heavy bacterial suspension, is a simple and rapid means of differentiation.

Pathogenicity for Man.[10]—Experimental evidence and that of comparative pathology show that man is more susceptible than the ordinary laboratory animals to staphylococcus infection. Garré[18] inoculated himself by rubbing a pure culture upon the uninjured skin of the forearm, with the result that a series of carbuncles was produced, seventeen scars remaining to testify to the success of the experiment. His experiments were repeated and confirmed by other workers.[19, 20] The penetration of the cocci into the deeper layers of the intact skin, probably through the sweat ducts or at the base of the hair follicles, is a fact of considerable significance. The positive occurrence of such penetration seems well established, and the negative observations of some authors may well be referred to differences in the virulence of the strains employed or to other experimental discrepancies.

The demonstration that staphylococci have power under certain circumstances to penetrate the skin, taken together with their practically constant presence upon the skin itself, serves to explain the multiplicity of human affections with which these microorganisms are found associated. A momen-

[13] *Cf.* Julianelle and Wieghard: Jour. Exp. Med., 1935, *62:*11, 23, 31.

[14] *Cf.* Fairbrother: Jour. Path. Bact., 1940, *50:*83.

[15] For details see Fisk: Brit. Jour. Exp. Path., 1940, *21:*311; Gillespie: Med. Res. Council (Great Britain) Monthly Bull., Emergency Pub. Health Lab. Service, 1943, *2:*19.

[16] Berger: Jour. Path. Bact., 1943, *55:*435.

[17] *Cf.* Cadness-Graves, Williams, Harper and Miles: Lancet, 1943, *i:*736.

[18] Garré: Fortschr. d. Med., 1885, *3:*165.

[19] Bockhart: Baumgarten, *Lehrbuch der path. Mykologie.* Braunschweig. 1890.

[20] Kaufmann: Baumgarten's Jahresb., 1900, *16:*110.

tary weakness on the part of the tissues in almost any locality may lead to a rapid local invasion, followed by the production of a simple boil or by a more or less extensive carbuncular condition. Septicemia and pyemia sometimes result through the introduction of staphylococci into the lymphatics or the blood stream from a local abscess. The initial lesion may be trivial in character. In the series of 122 cases studied by Skinner and Keefer[21] the case fatality rate was 82 per cent. Rapidly fatal bacteremia may occur without metastatic abscesses or metastatic abscesses may be produced, or the bacteremia may clear but leave focal abscesses and the infection still be fatal.

Staphylococci are not only found frequently in all parts of the body in secondary and mixed infections, but they are also primarily responsible for a variety of specific pathologic conditions and for injury to particular organs Many lesions and diseases of the skin have been attributed to staphylococci. In the case of some of these it has been claimed that special varieties or races are concerned, but the characters said to distinguish these from the ordinary *Staphylococcus aureus* or *albus* are not, as a rule, of differential value.

A considerable majority of all attacks of acute osteomyelitis and periostitis are due to staphylococci, which appear to have a special predilection for the tissues of the osseous system.

Suppurative inflammation, in whatever part of the body it may occur, is usually associated with the presence of staphylococci either in pure or mixed cultures. Sometimes when found in a mixed infection they are doubtless the original exciting cause; in other cases they may have arrived at the seat of trouble only after a primary invasion by some other bacterium. In a given instance it may be impossible to determine the precise sequence of events.

Staphylococcus infection of the lung sometimes occurs, and the resulting bronchopneumonia is often fatal. Out of about 800 patients with pneumonia treated at the Hospital of the Rockefeller Institute in New York City from 1913 to 1918, 13 were infected with staphylococci, and 10 of the 13 died. Under certain conditions, as during the 1918 influenza epidemic at Camp Jackson, *Staphylococcus aureus* may play an important part in the pneumonia complicating primary infections. Chickering and Park[22] found that in 49 per cent of 312 postmortem lung cultures this microorganism was present either alone (92 cases) or in association with other bacteria. Similarly, Gaspar[23] found that of 144 fatal cases of pneumonia cultured at autopsy, 38 were caused by staphylococci. In this series about two-thirds of the staphylococcus pneumonias occurred in the first decade of life. The presence of staphylococcus in the lungs is usually interpreted as a secondary invasion in the train of some primary exciting agent.

Staphylococcus food poisoning has been discussed in a previous chapter (Chap. 11).

Bacteriological Diagnosis.—The isolation of staphylococci from path-

[21] Skinner and Keefer: Arch. Int. Med., 1941, *68*:851.
[22] Chickering and Park: Jour. Amer. Med. Assn., 1919, *72*:617.
[23] Gaspar: New York State Jour. Med., 1941, *41*:834.

ologic material or from foods suspected in outbreaks of food poisoning is a simple matter. Blood agar is the medium of choice since the pathogenic strains are usually hemolytic *Staphylococcus aureus* and the hemolytic character is apparent on this medium. Examination of gram-stained smears from golden-yellow hemolytic colonies will show the characteristic morphology. Colonies may be picked for the coagulase test (see above) but fermentations are not significant and immunological typing is desirable only in special studies. The ability to form enterotoxin in the case of food poisoning strains may be demonstrated by feeding sterile filtrate of subcultures incubated in 25 per cent CO_2 to human volunteers in amounts of 2 to 5 cc., or to rhesus monkeys by stomach tube in 25 to 50 cc. amounts. The kitten test for enterotoxin is not reliable.

Pathogenicity for Lower Animals.—The rabbit has proved one of the more favorable animals for experimentation, intravenous injection of broth cultures being the most successful mode of infection. A moderately virulent strain kills an average-sized rabbit in four to eight days after injection of 0.1 cc. of a twenty-four hour broth culture. On autopsy, minute abscesses are found in various internal organs, most commonly in the kidney (particularly in the cortex) and in the walls of the heart. Under ordinary conditions of experiment with healthy adult rabbits the bone marrow and periosteum are rarely seriously affected. In young animals, however, several workers have claimed to have evoked typical osteomyelitis by intravenous injection of staphylococcus cultures. It is perhaps questionable whether in these cases the processes in the affected tissues are strictly comparable with natural osteomyelitis in man. The injection of cultures into a rabbit suffering from a fractured bone or an injured periosteum produces a more characteristic chain of events, and one that closely resembles the course of human osteomyelitis. Rabbits are relatively insusceptible to intraperitoneal inoculation with staphylococci. Artificial inoculation of the eye, on the other hand, succeeds readily, although natural eye infection is never observed. Feeding experiments with staphylococci do not produce infection. White mice are sometimes used for inoculation experiments, but are less uniformly responsive than rabbits; guinea pigs are relatively resistant, rats and pigeons highly so.

Cases of spontaneous staphylococcus infection among domestic animals, while not so common as in man, are not unknown. In horses and cattle *Staphylococcus aureus* has been found associated with pathologic processes and conditions similar to those that it produces in man. Staphylococcus mastitis in cattle is not uncommon.[24] Some observers believe that they have discovered special species of staphylococci in certain animal affections, such as "*Staphylococcus bovis*" (in cattle) and "*Staphylococcus hemorrhagicus*" (in sheep), but such species are of doubtful validity. Typical strains of *Staphylococcus aureus* and *albus* have been isolated from spontaneous abscesses in birds. A definite disease of the house fly has been traced to a specific staphylococcus (*Staphylococcus muscae*).[25]

[24] *Cf.* Minett: Jour. Comp. Path. Therap., 1937, *50*:101; Plastridge, Anderson, Williams and Weirether: Storrs Agr. Exp. Station Bull. No. 231, 1939.

[25] Glaser: Amer. Jour. Hyg., 1924, *4*:411.

Immunity.—An active immunity may be developed against staphylococcal infection by immunization with vaccines. In the rabbit the use of killed suspensions followed by living attenuated bacteria occasionally results in infection, and the inoculation of these microorganisms is somewhat dangerous. Immunization of human beings is, therefore, confined to the use of killed suspensions. The immunity so induced is in part antibacterial and in part antitoxic if the toxic products of the microorganism are included in the vaccine. The antibacterial immunity appears to be essentially a stimulation of the phagocytic mechanisms; the rate of phagocytosis is remarkably increased, and the activity of the phagocytic cells is of prime importance in combating infection with these bacteria. According to Valentine and Butler[26] antileucocidin is of considerable importance in the immunity. Antibacterial sera will protect untreated animals against infection, and the acquired immunity is associated with an increase in the amount of opsonin.

Fig. 40.—*Micrococcus tetragenus;* smear from pure culture stained with fuchsin. Note the relatively large size of the cells and the typical tetrad arrangement, with the irregular clumps tending to be made up of tetrads. × 1800.

The therapeutic use of vaccines in the treatment of boils and carbuncles and chronic furunculosis in man has met with some success. There is good evidence that the autogenous staphylococcus vaccine (the strain cultivated from the patient) is more efficacious than the ordinary stock vaccine.

In recent years considerable interest has attached to the antitoxic aspect of antistaphylococcus immunity. Toxoid, prepared by treating staphylococcus culture filtrates with formalin, has been used in producing an active antitoxic immunity, and a number of attempts have been made to demonstrate the efficacy of antitoxic sera. The therapeutic use of toxoid has given encouraging results in some instances, and it is of interest that the

[26] Valentine and Butler: Lancet, 1939, *ii:*973.
[27] *Cf.* Flaum: Acta. Path. Microbiol. Scand., Suppl. 35, 1938.
[28] *Cf.* Kleiger and Blair: Arch. Surg., 1943, *46:*548.
[29] Gaffky: Mitt. a. d. k. Gesund., Berlin, 1881, *1:*1.

antileucocidin appears to be of particular significance.[27] The value of antitoxic sera is uncertain. Their use, either through parenteral inoculation or local application, has not led to unequivocal results though in a number of instances very favorable results have been reported.[28] The antitoxin, it may be noted, is standardized in terms of antihemolysin, one unit being that amount which will neutralize 200 minimum hemolytic doses of toxin; in consequence the toxin is sometimes differentiated into α-toxin and β-toxin, referring to the α- and β-lysins.

OTHER MICROCOCCI

A variety of micrococci, both pigmented and otherwise, have been described, most of which are saprophytic forms found in water and elsewhere in nature. A well-known representative of this group is *Sarcina lutea*, a

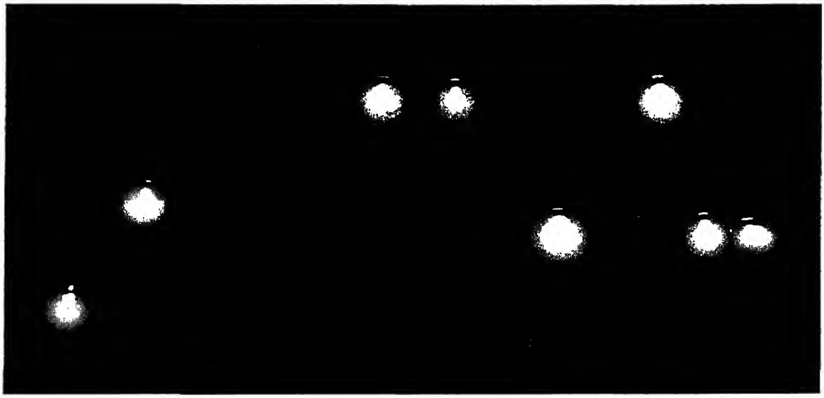


Fig. 41.—Colonies of *Micrococcus tetragenus* on blood agar. Twenty-four-hour culture. × 3.

coccal form producing a bright yellow pigment which derives its generic name from a tendency to form cubical packets of eight cells.

Micrococcus tetragenus (*Gaffkya tetragena*) is a parasitic coccus frequently found on the mucous membranes of the upper respiratory tract. It was discovered by Gaffky[29] in the pulmonary cavities in phthisis, and has been found in pure culture in abscesses in animals and man,[30] and often occurs in the healthy mouth. Morphologically *Micrococcus tetragenus* is distinguished by its occurrence in tetrads or groups consisting of four small oval cocci. It is gram-positive. In cultures the sheet-like arrangement is not always seen, but in the animal organism the flat tablets occur uniformly, and a rather heavy capsule surrounds the tetrad. On agar a confluent rough, elevated white growth is produced. On potato a thick, white, slimy growth occurs. Gelatin is not liquefied; milk is coagulated. Growth is slow and occurs at 20° and at 37° C., though better at the higher temperature.

[30] Müller: Wien. klin. Wchnschr., 1904, *17*:815.

White mice inoculated with *Micrococcus tetragenus* succumb to a rapidly progressing septicemia. Guinea pigs and rabbits usually show only a local affection. House mice and rats are relatively resistant. Fornaca[31] has reported a case of septicemia in man in which *Micrococcus tetragenus* was present in pure culture in the blood. It is not uncommonly found in suppurations of the mouth and neck. It is also found in the empyema following pneumonia and in the pus of war wounds.

This microorganism is probably of low-grade virulence, and unable, as a rule, to invade the human tissues except when the resistance is lowered by some depressing influence, especially of the kind caused by the invasion of some other bacterium.

[31] Fornaca: Rif. Med., 1903, *19*:309.

CHAPTER 15

THE STREPTOCOCCI

Family: *Lactobacteriaceae.* Tribe: *Streptococcaceae.* Genus: *Streptococcus.* Chiefly parasites. Cells normally in short or long chains (under unfavorable conditions, sometimes in pairs and small groups, never in large packets). Generally gram-positive. Capsules rarely present, no zoogleal masses. On agar streak, diffused translucent growth, often with isolated colonies. In stab-culture, little surface growth. Many sugars fermented with formation of large amount of acid, but inulin is rarely attacked. Generally fail to liquefy gelatin or reduce nitrates. Type species: *Streptococcus pyogenes.*

There is, in addition to the staphylococci, a large group of pyogenic cocci characterized by an arrangement of the cells in chains, which are responsible for a variety of human affections. The streptococci were early observed[1] in the pus formed during suppurative inflammation, but their constant presence and pathologic significance were first emphasized by Ogston,[2] Fehleisen,[3] and Rosenbach.[4] These microorganisms are found in nature in a variety of places, in water, milk, human and animal feces, and the healthy human throat, as well as in many diseased conditions of man and lower animals.

Morphology and Staining.—Individual streptococci, like staphylococci, are spherical, but differ from the latter in being usually united in longer or shorter chains. The diameter of the individual cocci is about 1 μ, although some variation is noted according to the character of the culture medium, and the cells are frequently considerably smaller in anaerobic cultures. Smaller varieties, one-half to two-thirds the diameter of the usual streptococci, have been described.[5] The typical streptococcus always divides in one plane, so that if the cells remain united a typical chain results. Transition forms between this and the staphylococcus have, however, been observed. Streptococci are not motile under ordinary conditions of observation, though motile species have been described,[6] and do not form spores. Most strains are encapsulated; in some cases the capsular material consists, in part at least, of hyaluronic acid (p. 183), and rare strains are pigmented.[7] They stain readily with the ordinary aniline dyes. Those isolated from pathologic processes in man are gram-positive, but some streptococci found in suppurative conditions in domestic animals are said to be gram-negative.

Some of the early investigators drew an important distinction between long-chain ("*Streptococcus longus*") and short-chain ("*Streptococcus brevis*")

[1] *Cf.* Thomson and Thomson: Editors of Annals of the Pickett-Thomson Research Laboratory, Vol. 3, Baltimore, 1927.

[2] Ogston: Brit. Med. Jour., 1881, *1*:369.

[3] Fehleisen: *Aetiol. d. Erysipels.* Berlin, 1883.

[4] Rosenbach: *Mikroorganismen bei d. Wundinfektionskrankheiten.* Wiesbaden, 1884.

[5] Long and Bliss: Jour. Exp. Med., 1934, *60*:619; Jour. Inf. Dis., 1938, *62*:52.

[6] *Cf.* Levenson: Ann. Inst. Pasteur, 1938, *60*:99 for a discussion of these forms.

[7] Wheeler and Foley: Proc. Soc. Exp. Biol. Med., 1942, *49*:421.

streptococci. The former were thought to be more virulent, and, as a matter of fact, the streptococci freshly isolated from disease in man usually grow out into long chains of more than eight cells, while saprophytic streptococci, such as are commonly found in the normal mouth and throat, develop short chains. Other morphological and biological characteristics were believed to correspond. It has been found possible, however, to transform the long-chain into the short-chain variety by alteration in the culture medium; growth on semisolid media, for example, is generally in the form of pairs and short chains, while the longer chains are formed in liquid culture. Short-chain streptococci which are virulent are sometimes isolated from pathologic processes. Any absolute distinction is, therefore, not possible, but the separation of long-chain and short-chain forms has some value as a generally valid correlation character.

Fig. 42.—*Streptococcus pyogenes.* Recently isolated scarlet fever strain. Note the tendency to diplococcus arrangement in the chains. Fuchsin; × 1050.

Streptococcus colonies on semisolid media are generally very small, translucent, convex, entire and slightly granular. Growth may be confluent in a transparent film, but there is a tendency for the colonies to remain discrete.

Physiology.—As a group, the streptococci will grow over a temperature range of 20° to 42° C. with an optimum at 37° C. This range is somewhat less for the pathogenic varieties. The majority of streptococci are facultative anaerobes, though there are some strictly anaerobic or microaerophilic varieties. The rôle of the anaerobic varieties in human infections is reviewed by McDonald, Henthorne and Thompson.[8] The streptococci are somewhat more fastidious in their nutritive requirements than some other bacteria, and growth on the nutrient (beef extract) media either is scanty or does not occur at all. The infusion media are, however, adequate, and growth may be enhanced by the addition of small amounts of glucose or of blood, ascitic

[8] McDonald, Henthorne and Thompson: Arch. Path., 1937, *23*:230; see also Stone: Jour. Bact., 1940, *39*:559.

fluid and similar substances. Many strains are hemolytic on blood agar, some showing the clear zone surrounding the colony of complete or β-hemolysis and others a zone of green discoloration termed α-hemolysis. The source of the blood is not important though human blood is reported to be less satisfactory than animal bloods.[9]

Growth occurs in milk, and is usually followed by curdling due to the fermentation of the lactose, but some strains, particularly the long-chain varieties, do not produce sufficient acid to precipitate the casein. Upon potato a more or less luxuriant growth is produced by most saprophytic forms, but the pathogenic varieties develop sparingly, and many refuse to grow at all. Streptococcus cultures are best preserved in serum broth or gelatin stabs in the icebox.

The complex nutritive requirements of the streptococci have not been defined with precision, but it appears that glutamine, riboflavine, pantothenic acid, vitamin B_6, and a variety of amino acids and probably other substances are required by these bacteria. Some strains have been cultivated in a synthetic or chemically defined medium.[10] Bernheimer and Pappenheimer[11] have developed a medium made up of hydrolyzed casein supplemented with amino acids, salts, glucose and vitamins of the B group which supports good growth of hemolytic streptococci.

A wide variety of sugars are fermented by the streptococci as a group but, with rare exceptions, *inulin is not fermented and these microorganisms are not dissolved by ox bile or a 10 per cent solution of bile salts*, characteristics of considerable practical significance, for they serve to differentiate the streptococci from the pneumococci. The hydrolysis of starch, esculin and sodium hippurate may or may not occur. The chief product of glucose fermentation is lactic acid, and small amounts of formic and acetic acids and ethyl alcohol are formed.[12]

Toxins.—The cell substance of streptococci possesses only slight toxicity, and virulent strains do not differ from avirulent in this respect. A variety of toxic substances may be found in streptococcus culture fluid, however, including hemolysins, fibrinolysin, leucocidin, spreading factor and an erythrogenic toxin.

At least two types of filterable hemolysin are formed by streptococci, the one sensitive to heat and acid and designated *streptolysin S*, and the other resistant to these but sensitive to oxygen and designated *streptolysin O*.[13] The inactivation of hemolysin by oxidation is reversible, and activity may be restored by mild reducing agents. The action of fibrinolysin, leucocidin and spreading factor have been discussed elsewhere (Chapter 8) and need not be further considered here.

[9] Dance and Murray: Jour. Inf. Dis., 1938, *63*:122.

[10] Hutchings and Wooley: Science, 1939, *90*:41. See also McIlwain *et al.*: Biochem. Jour., 1939, *33*:223; Hutner: Jour. Bact., 1938, *35*:429; King, Gary and Farrell: Jour. Bact., 1938, *36*:837; Subbarow and Rane: Jour. Amer Chem. Soc., 1939, *61*:1616; Wooley: Jour. Exp. Med., 1941, *73*:487.

[11] Bernheimer and Pappenheimer: Jour. Bact., 1942, *43*:481, 495.

[12] Friedemann: Jour. Bact., 1938, *35*:527; Jour. Biol. Chem., 1939, *130*:757; Smith and Sherman: Jour. Bact., 1942, *43*:725.

[13] Todd: Jour. Path. Bact., 1938, *47*:423.

The erythrogenic toxin is a substance which, upon intradermal injection in man, gives rise to a marked local erythema. Lower animals, with the possible exception of the rabbit, are not susceptible to this action, but it has been reported[14] that the lethal agent (for mice) produced in broth cultures cannot be distinguished from the erythrogenic toxin in properties and is neutralized by erythrogenic antitoxin. This toxin is responsible for the rash and systemic symptoms in scarlet fever and is also known as scarlatinal toxin or Dick toxin, after its discoverers (p. 330). It is antigenic, and induces the formation of antitoxin although not to the high titers that are found in diphtheria, tetanus and other antitoxic sera. It also differs from the other soluble toxins in that it is relatively heat-resistant, some toxicity still remaining in filtrates boiled for thirty minutes.

The production of these toxic substances is a property of streptococci as a group. Not all streptococci produce them, and toxin production is

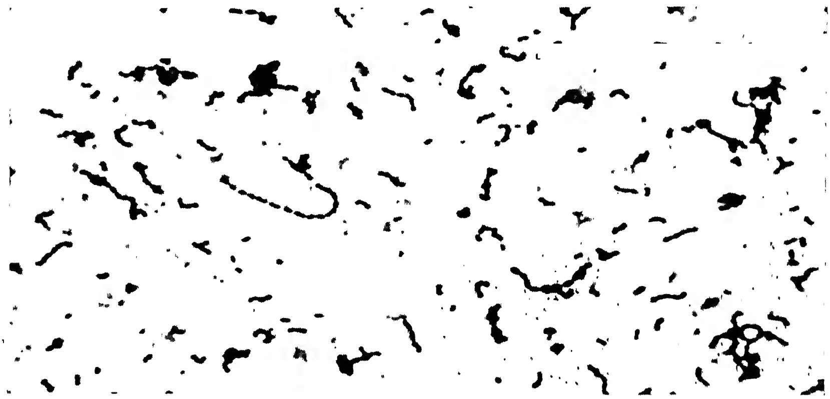


Fig. 43.—*Streptococcus fecalis.* P strain isolated from focal infection. Smear from pure culture. Fuchsin; × 1050.

roughly correlated with pathogenicity; saprophytic forms, for example, are frequently non-hemolytic, non-fibrinolytic and the like.

Variation.—Alterations in the morphology of the individual cells are frequently observed in old cultures; individual cocci may become swollen to several times their normal size before disintegration. These and other changes have been interpreted by some as indicating a complex life cycle for these bacteria (see Chapter 6). Dissociative changes in colonial morphology are also well known. Three colonial types were described by Todd[15]—in addition to the usual smooth and rough forms, an intermediate finely granular colony designated as "matt." It is of interest that the "matt" colony is the virulent form, both the smooth or "glossy" and rough forms being avirulent. Dawson has termed the "matt" form "mucoid" and has elucidated some of the immunological aspects of these dissociative

[14] Foley: Science, 1943, *98*:370.

[15] Todd: Brit. Jour. Exp. Path., 1928, *9*:91; Jour. Exp. Med., 1932, *55*:267.

changes.[16] According to Morison[17] the presence of capsules is associated with the type of colonial morphology.

Classification.[18]—Few groups of bacteria are so difficult to differentiate and classify as the streptococci. Blood plate hemolysis, biochemical characteristics and immunological relationships have been and are used in the differentiation of these microorganisms, and, although certain broad groups are observed, the separation of "types" and the definition of species are matters upon which there is by no means general agreement.

One of the most important of these criteria has been that of blood plate hemolysis, which was introduced by Schottmüller[19] and later subjected to elaborate study by Brown[20] and others.[21] On this basis three types of aerobic streptococci may be distinguished: (*a*) the β-hemolytic streptococci, grouped under the name *Streptococcus hemolyticus;* (*b*) the α-hemolytic or green-producing streptococci, grouped as *Streptococcus viridans;* and (*c*)

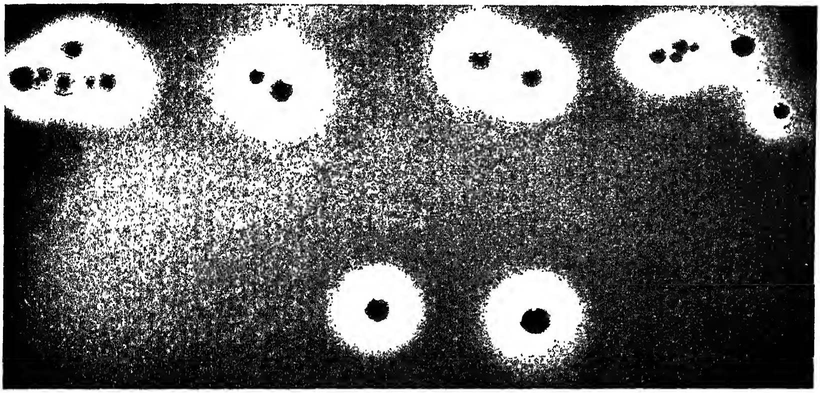


Fig. 44.—*Streptococcus pyogenes*. Pure culture on blood agar showing β hemolysis. × 5.

the indifferent or γ streptococci, termed *Streptococcus anhemolyticus*. Of these the hemolytic streptococci are, for the most part, those highly virulent forms associated with a variety of infections; the green streptococci, although producing disease, are considerably less virulent; while the nonhemolytic strains are generally saprophytes. These groups are broad and heterogeneous in that each is made up of a variety of different strains; hence a single species name, such as *Str. hemolyticus*, is too inclusive to be of real value.

[16] *Cf.* Dawson, Hobby and Olmstead: Jour. Inf. Dis., 1938, *62*:138.

[17] Morison: Jour. Path. Bact., 1940, *51*:401.

[18] The interrelationships of the streptococci are discussed at length by Sherman: Jour. Bact., 1938, *35*:81; Bact. Rev., 1937, *1*:1.

[19] Schottmüller: Münch. med. Wchnschr., 1903, *50*:849.

[20] Brown: *The Use of Blood Agar for the Study of Streptococci*. Monograph No. 9, Rockefeller Institute, 1919.

[21] Lyall: Jour. Med. Res., 1914, *30*:487; Holman: Jour. Med. Res., 1916, *34*:377; Blake: Jour. Med. Res., 1917, *36*:99.

Each of these broad groups may be subdivided into a series of species, types and varieties. Of the criteria that have been used, perhaps the least desirable is that of pathogenicity, which has led to the postulation of disease-specific types of streptococci such as *Str. scarlatinae*, *Str. epidemicus* (septic sore throat) and others, and there is little or no justification for the creation of such species. Two other criteria are used, usually to supplement one another, fermentation reactions and antigenic structure.

The Hemolytic Streptococci.—Greatest interest has centered about the β-hemolytic streptococci, for they are associated, for the most part, with diseases of man and lower animals. Although some use is made of cultural characteristics, emphasis has been placed primarily upon immunological methods for their differentiation. These immunological methods are of two types, first, agglutination and, second, precipitin tests with soluble antigens prepared from the bacterial cells.

INCIDENCE OF STREPTOCOCCI OF GROUPS A AND C IN HUMAN INFECTIONS*

Type of Infection	Number of Strains	Group A		Group C	
		Number	Per cent	Number	Per cent
Scarlet fever	232	229	98.7	3	1.3
Tonsillitis and septic sore throat	52	52	100.0	0	0
Rheumatic fever and rheumatic arthritis	19	19	100.0	0	0
Puerperal sepsis	55	51	92.7	4	7.3
Erysipelas	51	48	94.1	3	5.9
Miscellaneous	143	136	95.1	7	4.9
Total	552	535	96.9	17	3.1

* From Evans: Jour. Bact., 1944, *48*:267.

The main contributor to the differentiation of the streptococci on the basis of agglutination tests has been Griffith.[22] He has by this means differentiated and numbered twenty-seven types of pathogenic streptococci isolated from pathologic processes in man. Lancefield,[23] on the other hand, has made use of the precipitin test and has shown the presence of both group-specific and type-specific antigens in the hemolytic streptococci; *i.e.*, these bacteria fall into immunological groups as a consequence of the presence of a group-specific "C" substance which is a polysaccharide haptene, and the groups are in turn subdivided into immunological types by a type-specific "M" substance which is protein in nature. An additional antigen,

[22] *Cf.* Griffith: Jour. Hyg., 1935, *35*:23.
[23] Lancefield: Jour. Exp. Med., 1925, *42*:377, 397; *ibid.*, 1928, *47*:91, 469, 481, 483, 857; *ibid.*, 1933, *57*:571.

designated "T" substance, is also type-specific and differs from the "M" substance in that it is resistant to proteolytic digestion.[24] The biochemistry of these antigens has been reviewed by Kabat[25] and more recently studied by Lancefield.[26] The "M" and "T" substances are independent and may occur separately and in heterologous combination; thus the same strain of streptococcus may show more than one type specificity, *i. e.*, be a mixed type.[27] The agglutinogens of Griffith's types are these last antigens.

The biological significance of Lancefield's groups is indicated by the correlation of this immunological division with the natural habitat of the bacteria and by the relative biochemical homogeneity of the immunological groups.[28] Five groups were originally described:

Group A—pathogenic for man,
Group B—derived for the most part from cases of mastitis,
Group C—from various pathologic processes of lower animals,
Group D—isolated from cheese, and
Group E—isolated from milk.

Further investigation has confirmed this correspondence between group specificity and bacterial habitat and, in general, the hemolytic streptococci pathogenic for man are those of Group A, although there are occasional cases of infection with Group C. The animal pathogens are largely Groups B and C, and streptococci of the other groups are either saprophytic or nonpathogenic forms present in the intestine and other parts of the body.[29] To these five original groups others have been added which cannot be discussed at length here. A combination of both precipitin and agglutination reactions for typing is necessary for complete epidemiological studies.[30] While the original differentiation of these groups was carried out with β-hemolytic streptococci, not all strains which fall into them are β-hemolytic.

The Green-Producing Streptococci (Viridans Type).—The streptococci producing the green discoloration of α-hemolysis on blood agar differ from those of the β-hemolytic type in that a satisfactory subdivision by agglutination or precipitation methods has not been possible. Their differentiation is, then, possible only through fermentative and other biochemical characteristics. The viridans streptococci are, for the most part, of much lower pathogenic power than the β-hemolytic varieties, although at times highly virulent strains have been isolated. These streptococci are not infrequently found in blood culture in cases of subacute bacterial endocarditis and in focal infections of one sort or another, and are constant inhabitants of the intestinal tract of man and lower animals. The designation *enterococcus* is used by most French writers for the streptococci of the human intestine. In general, then, it may be said that the group of green streptococci con-

[24] Lancefield: Jour. Exp. Med., 1940, *71*:539.
[25] Kabat: Jour. Immunol., 1943, *47*:513.
[26] Lancefield: Jour. Exp. Med., 1943, *78*:465; *ibid.*, 1944, *79*:79.
[27] *Cf.* Elliott: Brit. Jour. Exp. Path., 1943, *24*:159; Watson and Lancefield: Jour. Exp. Med., 1944, *79*:89.
[28] *Cf.* Evans and Verder: Jour. Bact., 1938, *36*:133.
[29] For an account of the world-wide distribution of these groups see Rantz: Jour. Inf. Dis., 1941, *68*:128; *ibid.*, 1942, *71*:61.
[30] *Cf.* Krumwiede: Jour. Bact., 1943, *46*:117.

tains a number of microorganisms of potential pathogenic power as well as numerous saprophytic types.

The Indifferent Streptococci (Gamma Type).—Those streptococci which produce no change in the usual blood media are, in general, saprophytic forms which may be found in milk and various dairy products, and few if any of these are pathogenic for man or lower animals.

The classification of streptococci on the basis of hemolysis on blood agar, although a convenient procedure in many respects, is not altogether satisfactory, not only because each group is sufficiently heterogeneous that a single species name is not only undesirable but impractical, as noted above, but also because the characteristic of hemolysis cuts across other distinctions and groups certain varieties together that, by other criteria, are more closely related to members of other groups than to members of their own.

The Anaerobic Streptococci.—Streptococci of strictly anaerobic character may be found in the mouth, intestine and vagina and have been isolated occasionally from pathological lesions (*e.g.*, puerperal sepsis) in man. They are usually non-hemolytic and gas-forming. It may be noted that many of the commoner types of streptococci will grow under anaerobic conditions, and it is possible that these are the ones isolated from empyema and other suppurative conditions by anaerobic culture but later prove to grow aerobically.

Clearly Defined Species of the Genus Streptococcus.—As indicated above, there is no general agreement on this point, but, since some systematic approach is essential, the species proposed by Bergey[31] may be accepted in the tentative spirit in which they are offered.

A. The pyogenic group:
  1. Sodium hippurate −:
    (*a*) Lactose +:
      (1) Sorbitol −, trehalose +:
        Streptococcus pyogenes. β-hemolytic, Lancefield Group A. Includes all the important human pathogens.
      (2) Sorbitol +, trehalose −:
        Streptococcus sp. (animal pyogenes). β-hemolytic, Lancefield Group C. Various infections of lower animals.
    (*b*) Lactose −:
      Streptococcus equi. β-hemolytic, Lancefield Group C. Cause of strangles in horses.
  2. Sodium hippurate +:
    Streptococcus agalactiae (formerly *mastitidis*). β-hemolytic, Lancefield Group B. The most common cause of mastitis in cattle.

B. The viridans group. Since none of these green-producing streptococci is β-hemolytic, they are not included in Lancefield's groups.
  1. Lactose +:
    (*a*) Do not grow at 50° C.:
      (1) Starch −, do not survive 60° C. for thirty minutes, growth inhibited by bile:
        Streptococcus salivarius.[32] Found in subacute endocarditis, apical

[31] Bergey: *Manual of Determinative Bacteriology.* Williams & Wilkins Company. Baltimore. 5th ed., 1939.

[32] *Str. salivarius* ferments inulin but is distinguished from the pneumococci in that it is not bile-soluble.

abscesses of teeth and in general in the secretions of the mouth and upper respiratory tract.

(2) Starch +, survives 60° C. for thirty minutes, bile-tolerant:
Streptococcus bovis. Present in bovine mouth and intestinal tract, sometimes found in the human intestine.

(*b*) Grows at 50° C., no action on blood agar:
Streptococcus thermophilus. Found in milk and milk products, used as a starter in Swiss cheese.

2. Lactose −:
Streptococcus equinus. Probably not pathogenic, found in intestines of horses.

C. Lactic group:

1. Maltose +, dextrin +:
Streptococcus lactis. Not β-hemolytic, α-hemolysis variable. Found in milk and milk products. Not pathogenic.
2. Maltose −, dextrin −:
Streptococcus cremoris. No action on blood. Found in milk and milk products, used as a starter in butter and cheese manufacture.

D. Enterococcus group:

1. Do not liquefy gelatin:
(*a*) Mannitol +, sorbitol +, not β-hemolytic:
Streptococcus fecalis. Found in the human intestine and may be present in blood in endocarditis. Although not β-hemolytic, belongs to Lancefield's Group D.
(*b*) Mannitol −, sorbitol −, β-hemolytic:
Streptococcus durans. Found occasionally in milk products, belongs to Lancefield's Group D.
2. Liquefies gelatin:
(*a*) Variable α-hemolysis:
Streptococcus liquefaciens. Found in the human intestine and occasionally in subacute endocarditis.
(*b*) β-hemolysis:
Streptococcus zymogenes (regarded as a variety of *liquefaciens* rather than a separate species). Belongs to Lancefield's Group D.

There are, in addition to these, eight species of anaerobic streptococci which cannot be considered at length here. The relation of the streptococcus species to the Lancefield groups may be illustrated briefly:

Group A—*Streptococcus pyogenes*
Group B—*Streptococcus agalactiae*
Group C—*Streptococcus equi*, "animal pyogenes" and "human C"
Group D—the enterococcus species.

Pathogenicity for Man.—Few, if any, pathogenic microorganisms can lay claim to wider or more multifarious activities than the streptococci. The list of human diseases and affections with which streptococci are associated as the main and primary cause is already a long one, and is perhaps not yet complete. In addition to their conspicuous rôle as initiators of diverse pathologic conditions, streptococci are present in "mixed infections" and "secondary infections" more often than any other bacteria, *i.e.*, they have a tendency to follow in the wake of and act as accomplices to other pathogenic microorganisms. It has been found from an examination of the heart's blood of cadavers that in about one-third of all fatal diseases streptococci invade the blood before death, and in these cases they perhaps aid more or less in facilitating a fatal termination.

Of the β-hemolytic streptococci which invade the human body by far

the most common is *Str. pyogenes*, a designation which includes all members of Group A. *Str. pyogenes* is occasionally found in lower animals, but not infrequently its presence can be ascribed to human contamination. *Str. agalactiae* is essentially a pathogen of lower animals, as are also the streptococci of Group C. Man is, however, occasionally infected by streptococci of Group C, and these strains, designated as "Human C," are distinguished from *Str. equi* and "animal pyogenes" by trehalose and sorbitol fermentations. Members of other groups, although not important pathogens for man, are occasionally found. The green streptococci, although of lesser virulence, are not infrequently found in pathologic processes in man, particularly focal infections. According to Hucker[33] practically all of the strains in a collection of nearly 200 cultures of "*Streptococcus viridans*" obtained from clinical sources were found to be either *Str. salivarius* or *Str. fecalis*.

Suppurative inflammatory conditions in different organs of the body are, in many instances, caused by streptococci. Osseous tissues and tissues surrounding the bones are attacked less frequently by streptococci than by staphylococci, but the joints and serous membranes are often invaded. *Str. pyogenes* infection of the muscle, simulating gaseous gangrene, has been reported[34] and these organisms are frequently found in all types of wound infections. Infection with streptococci may, then, be generalized or localized in any part of the body and take a wide variety of clinical forms.

Focal infection is often due to streptococci, particularly of the viridans type. This name is given to a systemic or local disease due to bacteria carried in the blood or lymph stream from a focus of infection. The usual primary foci of infection are about the head, as in the nasal passages, the tonsils, the middle ear and the teeth. In the series studied by Macdonald, Simmons and Keogh[35] Group A hemolytic streptococci were isolated from 63 per cent of tonsils examined, and Groups B, C and G from 16 per cent. Carried from these primary foci, the streptococci may find lodgment in a distant organ. Appendicitis, iritis, and a variety of other diseases are probably sometimes caused by strains of streptococci carried in the blood stream from foci of infection in the tonsils, teeth or sinuses. The eradication of the primary foci of infection, such as removal of teeth or tonsils, has often been followed by rapid disappearance of long standing joint infections and other pathologic conditions.

Chronic arthritis or rheumatism is the most common chronic disease in temperate climates and far exceeds any other cause of chronic disability and suffering. Two clinical types are commonly recognized, the chronic atrophic and the chronic hypertrophic; infection is thought to play a larger part in the former than in the latter. The connection between an attack of tonsillitis and a coincident or subsequent attack of rheumatism has long been known, and the recognition of chronic focal infection as a cause of arthritic disease may be considered one of the great advances in medicine in this country. Elimination of the focus of infection, whether in

[33] Hucker: unpublished experiments quoted by Sherman: Bact. Rev., 1937, *1*:1.
[34] MacLennan: Lancet, 1943, *i*:582.
[35] Macdonald, Simmons and Keogh: Med. Jour. Australia, 1940, *1*:849.

the tonsils, a dental root-filling or elsewhere, is not infrequently followed by improvement, sometimes spectacular, in the joint conditions.

The bacteriology of chronic arthritis is confusing. Cultures of streptococci from a wide variety of sources and with diverse cultural characters are able to produce arthritis on inoculation into the joints of rabbits and other animals. Streptococci isolated from the normal throat seem to produce arthritis in animals as readily as those from the throats of arthritic patients. Strains of various kinds have been isolated from the tissues and from blood, and bacteria of other groups have sometimes been found. Hemolytic streptococci have been reported with considerable frequency, but green streptococci are also found, and in some instances those of the gamma type. Treatment with vaccines, usually of the hemolytic streptococcus type, has been widely practiced, but its efficacy is not established.

The analogy between arthritis and the allergic reaction produced by the sensitization of rabbits to streptococci has proved of great interest. In many instances infection does not result from a single joint injection but, if the animal is first sensitized by an injection of killed bacteria, an intravenous injection of the same type of streptococcus will produce arthritis. Such observations suggest that the relapses in *rheumatic fever* may, in some instances, be due to a reinvasion of the blood stream by the bacteria, provoking an allergic reaction in a primarily sensitized locus. The arthritic phenomenon may then conceivably be due in some cases to the sensitization of particular tissues by a streptococcus of relatively low virulence and a subsequent blood invasion by the same specific microorganism, and in others to direct primary infection with a streptococcus of high virulence which results in the acute condition, rheumatic fever. Whether streptococci are the sole cause of these affections is not certain, but it seems probable that they play an important rôle.[36]

Ulcerative endocarditis, an affection of the valves of the heart, which occasionally occurs simultaneously with inflammatory conditions in other parts of the body, is produced most frequently by streptococci, although it may also be caused by staphylococci, and less commonly by other microorganisms. Experimentally, the injection of broth cultures of streptococci (or staphylococci) into the circulation rarely produces ulcerative endocarditis unless the cardiac tissues have been damaged by mechanical or chemical agencies.

Streptococcus Sore Throat (*Septic Sore Throat*).—In the first decade of the twentieth century epidemics of sore throat of a severe and unusual type appeared in a number of localities in England and in the United States. The symptoms and complications have been strikingly similar in these and all other epidemics studied. Intense local hyperemia, with or without a grayish exudate, and enlargement of the cervical lymph nodes are among the more characteristic manifestations. The joints are affected in many cases and the heart and kidneys seriously damaged. Pneumonia ending in fatal septicemia is often observed, and peritonitis is not infrequently a cause of death.

The causative agent is a virulent β-hemolytic streptococcus, probably

[36] For example, see the review by Glover; Ann. Rheumatic Dis., 1942, *3*:4.

Str. pyogenes, although the earlier workers believed that a specific type of streptococcus was involved which was designated *Str. epidemicus*. Septic sore throat is frequently transmitted from man to man by contact, but the epidemics have been milk-borne. In some instances the milk can be contaminated directly by a human carrier of the streptococci, and in others the cow may be infected by man. It is probable that *Str. agalactiae* is not involved.

Bronchopneumonia and Secondary Infections.—Among the American troops in the first World War hemolytic streptococci were frequently found as the primary or secondary invaders in cases of bronchopneumonia. The numerous fatal cases of pneumonia following measles in the army camps seem to have been almost always due to infection with hemolytic streptococci. Many of these cases were accompanied by empyema in which hemolytic streptococci were likewise found. In certain localities the hemolytic streptococci, at first secondary invaders in the wake of the measles virus, seemed to acquire a higher degree of virulence and become able to initiate primary respiratory infection.[37]

The mucous membranes constitute a favorite abiding place for streptococci; the tonsils almost always harbor them. Consequently any lowering of the normal resistance of these tissues from either local or general causes gives the signal for a speedy invasion. In throat affections of all sorts streptococci are usually present, and in some cases, notably in diphtheria and in the anginas of scarlatina and measles, the part played by these bacteria is highly important. Typical diphtheria is so generally accompanied by a multiplication of throat streptococci that at one time these microorganisms were regarded as the cause of diphtheria. No one now believes that streptococci cause diphtheria, but there is little doubt that, as accessories to the diphtheria bacillus, they do considerable harm, and, in association with these bacteria, the virulence of the diphtheria bacillus appears to be enhanced (p. 138). As secondary invaders, streptococci may produce complications in the last stages of pulmonary tuberculosis, involve healthy tissues adjacent to the tuberculous area and predispose to hemorrhages. Streptococci are often present mixed with pneumococci in lobar pneumonia and in some cases act as the primary cause. In smallpox it is believed that many of the most serious symptoms and most frequent complications are the result of streptococcus infection.

Enteritis.—Certain cases of enteritis in infants and occasionally in adults have been attributed to streptococci, and in a number of instances streptococci have been implicated in food-poisoning outbreaks (p. 242) by epidemiological evidence. Bargen[38] has found a streptococcus in a series of cases of chronic ulcerative colitis which is similar to, if not identical with, *Str. fecalis*, but it is doubtful if there is any etiological relationship. In general, enteric infection with streptococci is rare and probably of no great significance.

[37] For example, see Lawrence and Sutliff: New York State Jour. Med., 1939, *40*:1233; Thomas: Bull. Johns Hopkins Hosp., 1943, *72*:218.

[38] *Cf.* Rankin, Bargen and Buie: *The Colon, Rectum and Anus*. W. B. Saunders Company, Philadelphia. 1932.

Erysipelas.—The pathogenicity of streptococci for man is well exemplified in erysipelas, a peculiar inflammation of the skin due to these bacteria. The cocci are not present in the central portion of the inflamed area, but are found on its periphery, and can be isolated most readily by excision of portions of the tissue, other methods rarely succeeding. In the skin they occur chiefly in the lymph spaces, which are often packed with them. The streptococci may be recovered by skin puncture as far as 3 cm. beyond the advancing edge of the lesion, where there is no gross evidence of inflammation. The inflammation is apparently not due to the erythrogenic toxin of Dick.

Erysipelas-like lesions in rabbits were produced by a number of early observers and have been extensively studied by later experimenters. Inoculation experiments made upon carcinomatous patients have also demonstrated that pure cultures of streptococci can provoke the erysipelatous process.

Immunity to erysipelas has been the object of a considerable number of studies because of its connection with the general problem of local immunity (p. 294). It seems established that treatment with immune sera exerts a mildly favorable effect upon the immediate attack in the majority of cases, but does nothing to ward off recurrences. Complications, such as abscesses, are not diminished in number but are possibly reduced in severity. It is of some interest that a local passive immunity may be obtained by infiltration of the skin with antiserum.

As in the case of septic sore throat, it has been thought by some that the erysipelas streptococci constitute a definite species, *Str. erysipelatis*, but, as will appear, this supposition is not justified. Streptococci other than the β-hemolytic forms are apparently not involved.

Streptococcic Septicemia.—In a great variety of affections, both those of streptococcal etiology and those caused by other microorganisms, streptococci may gain entrance to the blood stream and cause a septicemic condition in which there may be localization of the invading microorgansms in the joints, subcutaneous tissue, endocardium and elsewhere. The case-fatality rate in streptococcus septicemia is high, and recovery is associated with the development of antibodies in the blood. In a study of 246 patients it was found[39] that the case fatality was 72 per cent and about 30 per cent of the patients showed metastases. It may be noted that a transient bacteremia may develop following the excision of infected tissue such as tonsils.[40]

Puerperal sepsis, or childbed fever, is generally due to β-hemolytic streptococci, although other bacteria are occasionally implicated. The source of infection may be exogenous or endogenous. The once common hospital epidemics of puerperal septicemia were probably due largely to the manipulative transfer of streptococci from infected cases. Endogenous infection may, however, take place, for hemolytic streptococci may be found in the vaginal secretions of 1 to 3 per cent of pregnant women, and the anaerobic streptococci normally found in the vagina have been implicated in a number

[39] Keefer, Ingelfinger and Spink: Arch. Int. Med., 1937, *60:*1084.
[40] *Cf.* Elliott: Lancet, 1939, *ii:*589.

of cases of puerperal sepsis. It is generally believed that streptococci present in the vagina are of low virulence and cause infection only when conditions are particularly favorable for their growth, such as the occurrrence of severe lacerating injuries and the like. The source of infection is, for the most part, exogenous.

Scarlet Fever.—Although it had long been observed that β-hemolytic streptococci were almost invariably associated with scarlet fever, most workers hesitated to believe that this disease was of streptococcal etiology, in part because of the ubiquitous distribution of β-hemolytic streptococci in the human throat and their importance as secondary invaders, and in part because of the relatively lasting immunity left by an attack of the disease as contrasted with the low and transient immunity conferred by many streptococcus infections. In 1923, however, the Dicks[41] succeeded in reproducing typical scarlet fever in human volunteers by the inoculation of pure cultures of β-hemolytic streptococci, and in the following year Dochez and Sherman[42] evoked significant symptoms in guinea pigs and shoats by the inoculation of streptococci into agar injected subcutaneously. The Dicks[43] also showed that the filtrate of cultures that had produced experimental scarlet fever, when injected intracutaneously in the proper dilution, gave a distinct local reaction in the skin of a large proportion (41.6 per cent) of persons who had no history of scarlet fever, while all of the convalescent scarlet fever patients tested showed negative or only slightly positive reactions. It was further found that the action of the filtrate on the skin was inhibited by small quantities of convalescent scarlet fever serum mixed with the filtrate before injection.

The observations of the Dicks on the formation of erythrogenic toxin by streptococci and the essential specificity of the skin test have been confirmed and extended by numerous workers in various parts of the world. These observations, together with the already well-known association of streptococci with scarlet fever, have definitely established the streptococcal etiology of this disease.

Scarlet fever is a clinical entity in that a rash, produced by the erythrogenic toxin, is associated with the streptococcal infection of the throat. Invasion of other parts of the body is not uncommon and results in the well-known sequelae of the disease such as middle ear infection, nephritis, etc. Immunity to scarlet fever is, in a sense, a clinical immunity in that it is largely an immunity to the Dick toxin rather than to the streptococcus. It may be demonstrated by the *Dick test*, a skin test analogous to the Schick test in diphtheria, *i.e.*, the local erythema is due to the action of the toxin and is absent in the presence of antitoxin, either of exogenous origin or present in the immune individual. The Dick test may be used, then, to ascertain whether or not an individual is immune to scarlet fever or, more precisely, whether circulating antitoxin is present. In this connection it is of interest that Schultz and Charlton[44] earlier observed that when a scarlet

[41] Dick and Dick: Jour. Amer. Med. Assn., 1923, *81*:1166.
[42] Dochez and Sherman: Jour. Amer. Med. Assn., 1924, *82*:542.
[43] Dick and Dick: Jour. Amer. Med. Assn., 1924, *82*:265.
[44] Schultz and Charlton: Ztschr. f. Kinderheilk., 1918, Orig. *17*:328.

fever patient with a bright-red rash is injected with 1 cc. of convalescent serum, after about six hours the rash begins to fade and soon disappears completely. The significance of this phenomenon, the *Schultz-Charlton blanching phenomenon*, was not recognized at the time.

The Erythrogenic Toxin.—The erythrogenic toxin differs from the other soluble bacterial toxins in that it is resistant to heat, as noted earlier. Its nature is not clear as yet, however. It has been reported to be a protein by some workers and a polysaccharide-containing complex by others.[45] More recently Barron, Dick and Lyman[46] have reported that it is a protein of low molecular weight and is resistant to proteolytic digestion and a number of oxidizing and reducing agents. Stock[47] has prepared a highly purified heat-coagulable protein containing 10^7 STD per milligram which he regards as identical with the erythrogenic toxin. Koerber and Bunney,[48] however, have reported the isolation of a protein-free toxin. Four components have

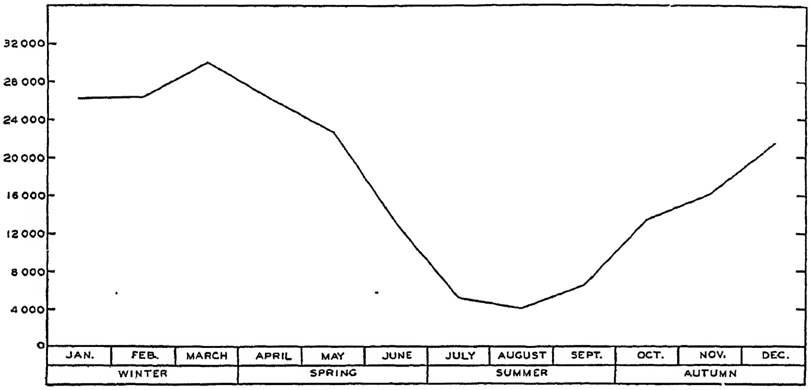


Fig. 45.—The seasonal incidence of scarlet fever. Averages of reported cases by months for the years 1930 to 1938 inclusive. Data from Supplements to Public Health Reports.

been demonstrated by electrophoresis; the toxicity was associated with the slowest and on separation this contained 10^8 STD per milligram.[49] Whatever its nature, it is necessarily assayed by skin test and its potency is measured in skin test doses (STD), the smallest amount that will produce the characteristic erythematous response. It is, of course, antigenic, and is flocculated by antitoxic sera.[50]

It has been held by the Dicks and others, largely on the basis of early agglutination studies, that the scarlet fever streptococci constitute a homogeneous group of β-hemolytic streptococci which should be designated *Str.*

[45] Earlier work is reviewed by Eaton: Bact. Rev., 1938, *2*:3.
[46] Barron, Dick and Lyman: Jour. Biol. Chem., 1941, *137*:267.
[47] Stock: Jour. Biol. Chem., 1942, *142*:777.
[48] Koerber and Bunney: Jour. Immunol., 1941, *40*:459.
[49] Krejci, Stock, Sanigar and Kraemer: Jour. Biol. Chem., 1942, *142*:785.
[50] Hottle and Pappenheimer: Jour. Exp. Med., 1941, *74*:545; Proom: Jour. Path. Bact., 1941, *53*:39.

scarlatinae. While the streptococci isolated from scarlet fever are all members of Group A, the ability to produce erythrogenic toxin is not confined to particular immunological types within this group; hence these microorganisms are not immunologically homogeneous. Neither are they biochemically homogeneous; in some of the Dicks' early experiments, for example, scarlet fever was produced with some strains that fermented mannite and with others that did not. It appears, therefore, that the scarlet fever streptococci are strains of *Str. pyogenes* that have in common the ability to form erythrogenic toxin. These strains are not necessarily associated with clinical scarlet fever, however, and may be found in erysipelas and other streptococcal infections of man. This is, of course, a matter of primary importance in any consideration of the epidemiology of scarlet fever.[51]

Scarlet Fever Antitoxin.—The therapeutic use of antistreptococcus sera was investigated by Moser[52] in 1902 with encouraging results, and similar observations were reported by Dochez and others.[53] The Dicks prepared specific antitoxic sera by immunizing horses with sterile culture filtrates. This antitoxic serum, in common with the earlier antibacterial sera, produces the Schultz-Charlton phenomenon. The results of the therapeutic use of the Dick antitoxin are favorable on the whole, though the mildness of the prevalent type of scarlet fever makes it difficult to secure any large body of statistics as cogent as those recorded in diphtheria. In individual cases, however, the administration of antitoxin decreases the duration of the rash, changes the character and extent of desquamation and reduces the number of complications, and there is general agreement as to the efficacy of antitoxic serum properly prepared and administered early. Some clinicians would restrict the use of scarlet fever antitoxin to cases of severe or toxemic type. A unit of antitoxin is defined as that amount which will neutralize 50 skin test doses of toxin.

There is some evidence that certain diseases of streptococcal etiology, including some of those that are clinically scarlet fever, are more directly due to the substances closely bound to the body of the bacterial cell (endotoxins) than to the diffusible rash-producing exotoxin. Little is yet known about these substances, and the serum treatment of the bacteremic type of many streptococcus infections is still in an elementary stage.

Prophylactic Inoculation.—It is not generally known that following Jenner's work on smallpox vaccination (p. 6) attempts were made to immunize against scarlet fever by a similar process of inoculation.[54] Preventive inoculation with killed streptococcus cultures was practiced by Russian bacteriologists as early as 1906.[55] Mild symptoms were produced similar to those observed in scarlet fever. A single injection did not suffice to produce

[51] For a detailed field study of the epidemiology of scarlet fever see Schwentker, Janney and Gordon: Amer. Jour. Hyg., 1943, *38:*27.

[52] Moser: Wien. klin. Wchnschr., 1902, *15:*1053.

[53] Dochez: Proc. Soc. Exp. Biol. Med., 1924, *4:*184; Blake, Trask and Lynch: Jour. Amer. Med. Assn., 1924, *82:*712.

[54] Dick and Dick: *Scarlet Fever.* Year Book Publishers. Chicago. 1938.

[55] Gabritschewsky: Centralbl. f. Bakt., 1906, I: Orig., *71:*719; Nikitin: Jour. Amer. Med. Assn., 1926, *87:*2143.

immunity, two or three inoculations being necessary. It was believed that a considerable degree of immunity was obtained by this procedure. Korschun and Spirina,[56] using a formalinized vaccine, reported good results.

The discovery of the scarlet fever toxin offered an opportunity for protective immunization similar to that successfully utilized in diphtheria, and was early taken advantage of by the Dicks.[57] A standard toxin is necessary for the safe and effective application of this method. Toxins with a potency of at least 40,000 STD per cubic centimeter are preferable, and appropriate dilutions are injected at intervals of one week. Five injections are recommended, starting with 500 STD and gradually rising to about 100,000 STD. Immunization of susceptibles (Dick positives) in this manner produces 98 per cent or more Dick negatives. The injections are sometimes accompanied by the development of a scarlatiniform rash and other symptoms of mild scarlet fever. In consequence, the use of toxin detoxified by treatment with formalin has been advocated by some workers, but the efficacy of such prep-

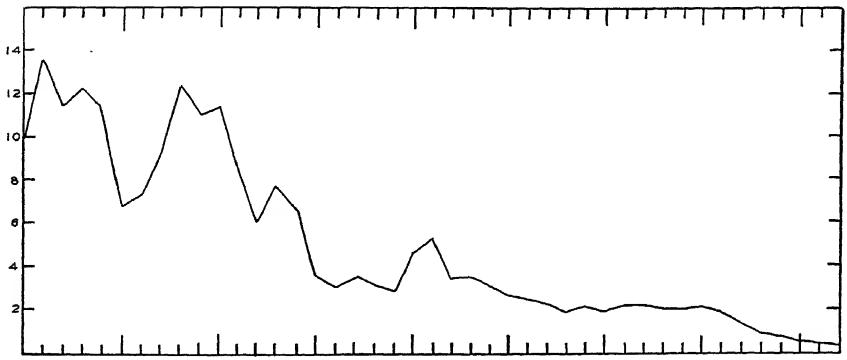


Fig. 46.—The prevalence of scarlet fever in the Registration Area since 1900 as indicated by the uncorrected death rates. From data compiled by the Bureau of the Census.

arations has been denied by the Dicks.[58] Veldee[59] has reported that a purified precipitated toxin apparently produces a good immunity with fewer untoward reactions. It is to be emphasized, however, that immunity to scarlet fever is an immunity to clinical scarlet fever, not to the streptococcal infection, and from the epidemiological point of view both Dick-negative and Dick-positive individuals must be considered as having scarlatinal infections, the only difference being the clinical one dependent upon the development of a rash.

As in the case of diphtheria, immunity to scarlet fever may be acquired by inapparent infection (p. 565). The frequency of positive Dick tests is low in newborn children (indicating a passive immunity of maternal origin),

[56] Korschun and Spirina: Seuchenbekämpfung, 1927, *4*:40.
[57] Dick and Dick: Jour. Amer. Med. Assn., 1924, *82*:544.
[58] *Ibid.*, 1934, *103*:1362.
[59] *Cf.* Veldee, Peck, Franklin and DuPay: Pub. Health Repts., 1941, *56*:957.

then rises to a maximum in the age groups one to five years, and thereafter falls off gradually until in persons over thirty it is relatively low, possibly 15 per cent.

The prevention of scarlet fever by specific immunization is an effective procedure, and favorable results have been reported from various parts of the world. Immunization is, however, not nearly so common as in the case of diphtheria and smallpox, for, unlike these diseases, other methods of prevention are effective. Milk-borne scarlet fever, for example, is prevented by adequate sanitary control including pasteurization, and, although scarlet fever is transmissible from man to man, the decline in the death rate from this disease in the last four decades (Fig. 46) is largely attributable to a general hygienic improvement in living conditions.

Bacteriological Diagnosis of Streptococcal Infection.—The isolation of streptococci from specimens of pathological material is ordinarily not difficult. An enriched medium is required and blood agar is the medium of choice for both α-hemolysis and β-hemolysis are apparent. Overgrowth by *Proteus* in cultures of some kinds of specimens may be prevented by including 0.02 per cent sodium azide in the medium. Most specimens, such as throat swabs, pus, etc., may be streaked directly on blood agar but enrichment culture, as in veal infusion broth containing 0.1 per cent dextrose and 0.1 per cent phosphate buffer, should be made with blood taken for culture, incubated for twenty-four hours, and then streaked on blood agar. If there is reason to believe that the specimen contains sulfa drug, its bacteriostatic effect may be neutralized by including 5 mg. per 100 cc. of *p*-aminobenzoic acid in the medium.

The colonial morphology is typical in the case of β-hemolytic streptococci and the characteristic chains of cocci may be found in gram-stained smears. Green streptococci from sputum and similar specimens must be differentiated from pneumococci by inulin fermentation and bile solubility. The hemolytic streptococci may be typed by agglutination with type specific antisera and by the precipitin test. For the latter the sedimented bacteria from a 250 cc. broth culture are suspended in 10 cc. of N/10 HCl in saline, boiled for ten minutes, cooled in running water, and the insoluble material spun out to leave a clear supernatant to be used as the antigen. Considerable economy of reagents may be effected by setting up the precipitin test as a ring test in capillary pipettes; the precipitate at the serum-antigen interface may be observed with a hand lens.[60]

Pathogenicity for Lower Animals.—The outstanding diseases of streptococcal etiology in lower animals are strangles in horses (*Str. equi*) and mastitis in cattle (*Str. agalactiae*). A variety of suppurative processes, focal infections and septicemias in domestic and experimental animals may be observed. They are less common than simple staphylococcus infections but, as in man, comprise perhaps one fourth of all local infections. As indicated previously, the β-hemolytic streptococci found in lower animals usually belong to Groups B and C, and *Str. pyogenes* is probably only very rarely present under natural conditions.

Immunity.—Immunity to streptococcus infections is, in general, of low

[60] Swift, Wilson and Lancefield: Jour. Exp. Med., 1943, *78*:127.

order and in any case transient, the exception being antitoxic immunity to scarlet fever. In consequence, active immunization against streptococcus infections is usually unsatisfactory, although a few workers have reported a significant increase in resistance. There is some evidence that immunity is specific to agglutinin type but there is also a certain amount of cross-protection.[61] Streptococcus infection is not infrequently associated with the development of antibodies such as antistreptolysin, agglutinin and others, but phagocytic activity is weak as contrasted with the staphylococcus infections, and the observable antibodies do not appear to exert a significant protective action. It follows, of course, that serum therapy of streptococcus infections is usually ineffective.

Disease-Specific Streptococcus Types.—The existence of disease-specific types of streptococci has been frequently postulated at one time or another, and names such as *Str. erysipelatis*, *Str. epidemicus*, and *Str. scarlatinae* have crept into the literature as noted above. As may be inferred, however, there appears at present to be no justification for such a subdivision of the streptococci of Group A (*Str. pyogenes*). In certain areas and within limited periods of time there is some tendency for recurrence of streptococcus types. For example, Schwentker and his co-workers[62] observed that Types 1 and 6 were generally associated with pharyngitis and tonsillitis and Types 3 and 19 with scarlet fever in a naval training station. Similarly, Hilles, Hamburger, Johnson and Wallin[63] found that scarlet fever was associated with Types 3, 17 and 19 one year, but the previous year Type 18 had been involved for the most part. Such associations are attributable in part to the prevalence of particular strains and to their relative virulence and toxigenicity. It is well known that such diverse pathological conditions as scarlet fever, erysipelas, tonsillitis, cervical adenitis and otitis media can be caused by identical or closely related streptococci[64] and, conversely, that a single disease, such as scarlet fever, can be caused by many immunological types of streptococci.[65] Any streptococcal infection may be regarded as but a single manifestation of a large group of diseases due to the same infecting agent. Whatever importance this may have in the academic sense, its practical significance is obvious in connection with the transmission of streptococcus infection; a streptococcus sore throat in one individual may be, and not infrequently is, scarlet fever in another. Two diseases of lower animals, mastitis and strangles, are, however, definitely associated with specific types of streptococci, *Str. agalactiae* and *Str. equi* respectively, and these streptococci are, to a considerable degree, disease-specific.

[61] *Cf.* Evans: Jour. Immunol., 1941, *42*:15; *ibid.*, 1943, *46*:399.
[62] Schwentker *et al.:* Amer. Jour. Pub. Health, 1943, *33*:1454.
[63] Hilles, Hamburger, Johnson and Wallin: Jour. Amer. Med. Assn., 1944, *124*:564.
[64] *Cf.* Boisvert *et al.:* Amer. Jour. Dis. Children, 1942, *64*:505, 516.
[65] *Cf.* Bynoe: Canadian Jour. Pub. Health., 1943, *34*:272.

CHAPTER 16

THE PNEUMOCOCCI

Family: *Lactobacteriaceae*. Tribe: *Streptococcaceae*. Genus: *Diplococcus*. Parasites. Grow on the rich artificial media. Somewhat elongated cells, usually in pairs, sometimes in short chains, often encapsulated. Gram-positive. Active fermenters, most strains forming acid in dextrose, lactose, sucrose and inulin. Facultative anaerobes, some species strict anaerobes. Type species: *Diplococcus pneumoniae*.

The bacterium most commonly found in pneumonia in man is a small lanceolate micrococcus which has been variously termed *Micrococcus pneumoniae*, *Micrococcus lanceolatus*, *Streptococcus pneumoniae*, or more briefly, the *pneumococcus* or *Fränkel's pneumococcus*. *Diplococcus pneumoniae* is now the commonly accepted formal name.

THE INCIDENCE OF ETIOLOGIC AGENTS IN PNEUMONIA*

Causative Organism	Lobar Pneumonia	Broncho-pneumonia	Unspecified	All Pneumonias
Pneumococcus	82.48	65.79	77.48	77.71
Hemolytic streptococcus	2.00	3.33	3.99	2.65
Other streptococci	1.30	2.99	1.33	1.70
Staphylococcus	.82	2.00	1.38	1.19
Friedländer's bacillus	.15	.13	.28	.17
Influenza bacillus	.06	.25	.11	.15
Tubercle bacillus	.....	.08	.....	.02
Fungi	.....	.02	.....	.....
Virus	.....	.....	.07	.01
No significant organism recorded	13.19	25.41	15.38	16.44
Number of cases	15,420	6,092	4,290	25,802

* In six representative states over a two-year period as compiled by Rumreich *et al.*. Pub. Health Repts., 1943, *58*:121.

Of the generally recognized anatomical types of pneumonia, lobar or acute croupous pneumonia, bronchopneumonia or lobular pneumonia, and capillary bronchitis or bronchiolitis, lobar pneumonia is nearly always due to the pneumococcus, though other bacteria are occasionally involved. Perhaps the best quantitative data are those assembled by Rumreich and his co-workers[1] in a two-year study in six states representing high and low pneumonia rates. These are summarized in the accompanying table. It will be clear that while the pneumococcus is by far the commonest cause of pneumonia, other bacteria are somewhat more frequent in bronchopneumonia than in lobar pneumonia.

[1] Rumreich *et al.*: Pub. Health Repts., 1943, *58*:121.

The pneumococcus was discovered independently by Pasteur[2] in France, who inoculated rabbits with the saliva of a child dead of rabies, and by Sternberg[3] in the United States, but was not known to be associated with disease in man before the extensive investigations of Fränkel[4] and Weichselbaum,[5] who demonstrated conclusively the etiological relation of this bacterium to pneumonia in man.

Morphology and Staining.[6]—The pneumococcus is typically a small, slightly elongated coccus, one end of which is pointed or lance-shaped. The cocci commonly occur in pairs (diplococci), but variations both in grouping and in size and form of individual cells are frequently observed. Chain formation is common, especially in artificial media, although the chains are usually shorter than those of *Streptococcus pyogenes*. Oval and elongated bacillary forms sometimes occur. The pneumococcus is non-motile and does not form spores. A well-defined capsule envelops the pneumococci in animal

Fig. 47.—Pneumococcus, pure culture. Note the typical lanceolate shape and diplococcus arrangement. Fuchsin; × 1050.

exudates but, except in certain strains or in certain media, is less readily demonstrable in cultures grown outside the body. Capsules may often be found in milk cultures and in media containing blood or serum.

The pneumococcus is readily stained with the aniline dyes and is generally gram-positive, although there is a tendency to become gram-negative in older cultures and occasional strains are found to be gram-negative. In stained preparations the capsule may often be seen as an unstained halo surrounding the cells; it may be stained by special methods.

The colonies of pneumococcus on infusion agar or blood agar are typically

[2] Pasteur: Compt. Rend. Acad. Sci., 1881, *92*:159.

[3] Sternberg in National Board of Health Annual Report, 1881, p. 87.

[4] Fränkel: Verhandl. d. Cong. f. innere Med., Wiesb., 1884, *3*:17; Ztschr. f. klin. Med., 1886, *10*:401, 426; *ibid.*, 1886, *11*:437.

[5] Weichselbaum: Med. Jahrb., 3rd ser., 1886, *1*:483.

[6] The pneumococcus is completely and exhaustively discussed by White: *The Biology of Pneumococcus*. The Commonwealth Fund. New York. 1938.

small, moist, translucent and granular, with well-defined edges. These bacteria are α-hemolytic on blood agar, and the colonies appear surrounded by a zone of greenish discoloration on this medium and resemble the colonies of green-producing streptococci.

Physiology.—Some pneumococci grow upon the ordinary nutrient (beef extract) culture media but many do not, and in any case growth is sparse. Nutritional requirements are complex; Rane and Subbarow[7] have been able to grow Types I, II, V and VIII but not Type VII in a medium consisting of acid hydrolysate of gelatin supplemented with glutamic acid, cystine, glucose, pantothenic acid, nicotinic acid, choline and thioglycollic acid. Growth on infusion media, particularly those enriched by the addition of whole blood, takes place at 37° C. Litmus milk is promptly acidified and often, but not invariably, coagulated. The temperature range over which these bacteria may be grown is relatively narrow (25° to 42° C.), and they are sensitive to variations in pH from the optimum of 7.8, the limiting

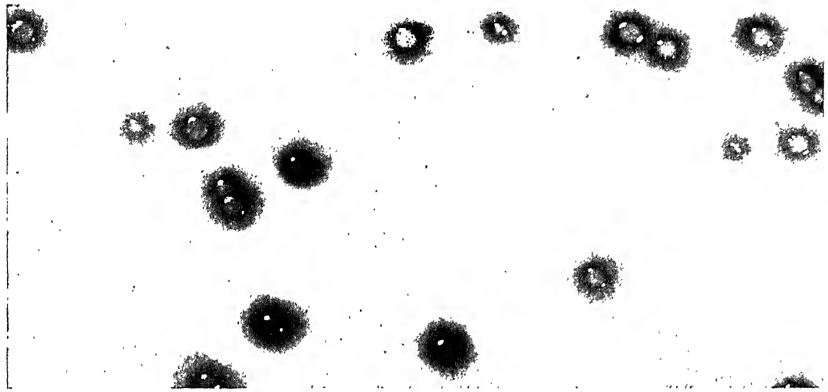


Fig. 48.—Colonies of the pneumococcus on blood agar. The areas of green hemolysis have been accentuated in the photograph. × 3.

acidity and alkalinity being 7.0 and 8.3 respectively. The pneumococcus is a facultative anaerobe, although certain other species of *Diplococcus* are obligate anaerobes.

In general, sugars are actively fermented with the production of large amounts of lactic acid and small amounts of volatile acid and ethyl alcohol. Differential fermentations are of no particular value in the classification of these microorganisms except in the case of inulin, which serves to differentiate the pneumococci and the green streptococci.

The pneumococci appear to be structurally delicate organisms and autolyze much more readily than most other kinds of bacteria. Evidences of protein hydrolysis accompany autolysis, and it appears that lysis is a consequence of the activity of intracellular ferments. Perhaps associated with the autolytic process is the lysis of pneumococci by bile and bile salts. The

[7] Rane and Subbarow: Jour. Bact., 1940, *40*:695.

so-called "bile solubility" of pneumococci is practically a constant characteristic, although different strains vary in their sensitivity to bile as they do in the tendency to autolysis. Rabbit or ox bile may be used and added to a young broth culture in the proportion of 10 to 20 per cent. Solutions of pure bile salt (sodium taurocholate) are preferable to ox bile because they may be sterilized and the concentration (10 per cent) controlled. Heat-killed pneumococci are not bile-soluble. Sodium lauryl sulfate and similar detergents will also lyse pneumococci.[8]

Pneumococci may, therefore, be distinguished from streptococci by their bile-solubility and, less definitely, their ability to ferment inulin, in addition to their greater pathogenicity for mice and the characteristics of the colonies on agar.

Peroxide is found in considerable quantities in pneumococcus cultures after prolonged incubation because of the lack of catalase in these microorganisms. This, coupled with their sensitivity to peroxide, results in the autosterilization of cultures kept in the incubator for many days. Cultures in blood broth, however, remain viable for several weeks in the refrigerator and the bacteria may be preserved for months in the cold in vacuum-desiccated spleens of infected mice.

The pneumococci are more sensitive to the bactericidal activity of the usual antiseptics than are many other bacteria. Soaps, such as ricinoleate and oleate, are pneumococcicidal in relatively high dilutions, 0.04 and 0.004 per cent respectively, and other substances such as phenol and mercuric chloride are also highly effective in the destruction of these bacteria. Quinine and some of its derivatives, such as optochin, are also pneumococcicidal, a fact which has been of interest in connection with the chemotherapy of pneumococcus pneumonia.

Toxins.—The severe intoxication observed in pneumococcus infection in man is suggestive of the formation of some toxin by this bacterium. The existence of such a toxin has never been demonstrated, however, and the pneumococcus does not produce a toxin analogous to those of the diphtheria and tetanus bacilli.

Other toxic substances are produced by this microorganism. That there is α-hemolysis on blood plates has already been noted, and there is in addition a filterable hemolysin active on sheep, guinea pig and human erythrocytes. The pneumococcus also produces a leucocidin and a necrotizing substance similar to that formed by some of the staphylococci. A purpura-producing substance which is non-antigenic and appears to be a cleavage product of pneumococcal protein has been described by a number of workers. Injected into white mice, extracts of pneumococci produce a purpuric condition manifested as a dark blue discoloration of the skin of the feet, tail, ears, nose and genitals.[9]

Although preparations containing these activities have been reported to increase the virulence of relatively avirulent pneumococcus strains when injected simultaneously with the bacteria, the virulence of pneumococci is

[8] Bayliss: Jour. Lab. Clin. Med., 1943, *28:*748; Harris and McClure: *ibid.*, 1942, *27:*1591.

[9] *Cf.* Julianelle and Reimann: Jour. Exp. Med., 1926, *43:*87; *ibid.*, 1927, *45:*609.

directly dependent, not on the formation of such toxic substances, but on the production of specific soluble substance and encapsulation.

Classification.—The pneumococci are closely related to the streptococci, but the degree of intimacy of the relationship is as yet open to question. Some workers regard these microorganisms as but a species of streptococcus and designate them as *Streptococcus pneumoniae*. In general, however, it is customary to consider the pneumococci as a distinct genus, a practice which is justified by considerations of the sum of the characteristics of the pneumococci and the clinical and epidemiological aspects of the pneumonias. According to Bergey's[10] classification, the tribe *Streptococcaceae* is made up of three genera: (1) *Diplococcus*, of which the type species is *Diplococcus pneumoniae* or the pneumococcus; (2) *Streptococcus*, with *Streptococcus pyogenes* as the type species; and (3) *Leuconostoc*, a group of gas-forming, chain-producing cocci found in milk, fermenting vegetables and slimy sugar solutions.

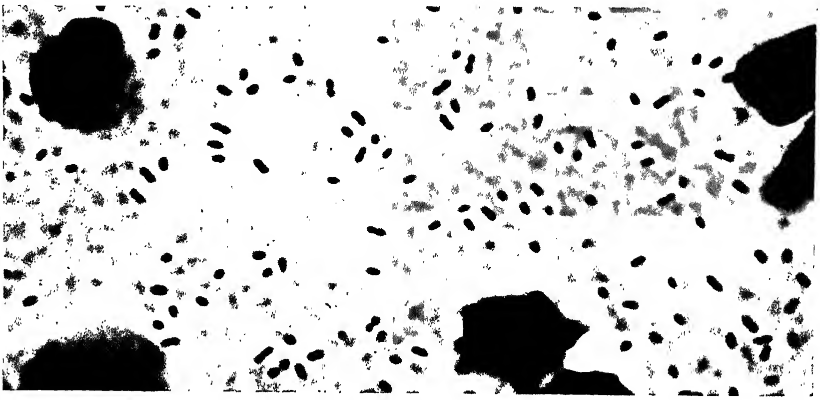


Fig. 49.—Pneumococcus in the peritoneal fluid of a mouse. Note the capsules. Fuchsin; × 2200.

Pneumococcus Types.—A point that has been of somewhat more interest than the formal taxonomic position of the pneumococci is the subdivision of these bacteria into types. The pneumococci contain two types of antigens. One, the so-called somatic antigen, is a constituent of the cell substance proper and is immunologically identical in all pneumococci. The other, the polysaccharide haptene or specific soluble substance (SSS), is type-specific and serves to differentiate the immunological types of pneumococci from one another. The polysaccharide of Type III has been the most thoroughly studied chemically (p. 249) and Types I and II to a lesser extent. These substances have been isolated from most of the other types also.[11] The presence of SSS masks the somatic antigen, and antisera to encapsulated pneumococci are sharply type-specific. These immunological

[10] Bergey: *Manual of Determinative Bacteriology*. Williams & Wilkins Company Baltimore. 5th ed., 1939.

[11] Brown: Jour. Immunol., 1939, *37*:445; Brown and Robinson: *ibid*., 1943, *47*:7.

types are designated by Roman numerals, and the numbering of the types, although purely arbitrary, is a universally accepted convention.

Observations by Dochez and Gillespie[12] showed that the pneumococci found in cases of lobar pneumonia can be divided into four distinct groups, designated as Types I, II and III and Group IV, on the basis of specific agglutination and protection tests. Of these, Types I, II and III, found in the majority of cases of pneumonia (Fig. 50) and producing the most severe types of infection, have specific immunological character, while Group IV is immunologically heterogeneous and is made up of all pneumococci not belonging to the first three types. Through the work of Cooper and her associates of the New York City Department of Health[13] 29 additional serologic types were sorted out of Group IV to make 32 types in all. Of these XXVI is closely related to VI and is sometimes designated VIa, and XV and XXX are identical or practically so. Six new types and 14 subtypes, related to but not identical with the Cooper types, were described by Kauffmann, Mørch and Schmith[14] who pointed out that the subtypes, while related, were stable and independent. This differentiation was made on the basis of reciprocal absorptions. Seventeen additional types were described by Walter, Guevin, Beattie, Colter and Bucca[15] of which 12 corresponded to 5 of the types and 7 of the subtypes of Kauffmann *et al.* Mørch[16] then later described additional new types, some of which were subtypes. The practice of designating subtypes, *viz.*, VIIa, VIIb, VIIc, and so on, has begun to be generally adopted. Under the auspices of the American Public Health Association and the United States Public Health Service, however, Eddy[17] has given individual type numbers to all pneumococcus types and subtypes so far described without regard to the relationships, sometimes very close, between many of the types, making a total of 75 types. Thus the purely arbitrary system of numbering the types without regard to their biological relationships is reverted to and extended.

Although these immunological types are readily distinguished from one another, cross-reactions occur in a number of instances. There appear to be some relations between Types II and V, III and VIII, VII and XVIII, and XV and XXX which are quite likely due to structural similarities of the polysaccharide haptenes (p. 252). The resemblances are not sufficiently close, however, to invalidate the differentiation of these types. An interesting observation has been that of Forster and Shaughnessy[18] of the occurrence of mixed types, *i.e.*, strains of pneumococci which react with two or more unrelated type-specific antisera. Similar observations have been reported by Chinn and Eddy.[19] Such mixed types appear to occur with some frequency as indicated in Fig. 50.

[12] Dochez and Gillespie: Jour. Amer. Med. Assn., 1913, *61*:727.

[13] Cooper, Edwards and Rosenstein: Jour. Exp. Med., 1929, *49*:461; Cooper, Rosenstein, Walter and Peizer: Jour. Exp. Med., 1932, *55*:531.

[14] Kauffmann, Mørch and Schmith: Jour. Immunol., 1940, *39*:397.

[15] Walter, Guevin, Beattie, Colter and Bucca: Jour. Immunol., 1941, *41*:279.

[16] Mørch: Jour. Immunol., 1942, *43*:177.

[17] Eddy: Pub. Health Repts., 1944, *59*:449, 451, 485.

[18] Forster and Shaughnessy: Proc. Soc. Exp. Biol. Med., 1940, *44*:306.

[19] Chinn and Eddy: Pub. Health Repts., 1941, *56*:62.

Diplococcus Mucosus.—These immunological types are culturally indistinguishable with the exception of Type III, which stands somewhat apart from the other pneumococci in that it produces a heavy mucoid growth due to its luxuriant capsule formation. It is not infrequently classified as a separate species, *Diplococcus mucosus.* Many cultures show a marked tendency to form chains, and the dividing line between *Diplococcus mucosus* and "*Streptococcus mucosus*" is not a sharp one, if indeed any distinction should be made. These coccoid, heavily capsulated bacteria for the most part ferment inulin and are soluble in bile, so that the tendency is to group them with the pneumococci rather than the streptococci. A few mucoid strains have been reported which are bile insoluble and non-inulin-fermenting.

Pneumococcus Typing.[20]—The determination of pneumococcus types is a matter of great practical importance to the therapeutic use of antiserum. The various methods that have been developed are basically immunological but differ in technical detail. The immunological procedures are of three kinds: (1) the agglutination of the pneumococci with type-specific antiserum; (2) the precipitation of SSS with type-specific antiserum; and (3) the *Quellung* reaction. The first two have been discussed in previous sections and need not be considered further here. The *Quellung* phenomenon was described by Neufeld in 1902[21] and redescribed in 1931,[22] and since the latter date has come into general use. A suspension of pneumococci is mixed with undiluted antiserum (rabbit serum is preferable to horse serum) on a slide or cover glass, a small amount of Löffler's alkaline methylene blue is added to facilitate observation, and the preparation is examined under the microscope. In the presence of homologous immune serum there is a marked apparent swelling of the capsule without any obvious change in the size of the bacterial cell itself; no such swelling is observed with heterologous sera. The reaction takes place rapidly and the swelling is usually apparent within a few minutes. The nature of the process of swelling is not known; it is of some interest that it may be reversed by the addition of homologous SSS.[23] The use of serum pools considerably facilitates typing, especially the identification of the higher types. The incidence of the various types determines the most advantageous combinations of antisera. A group of combinations often recommended for use in this country is: (*a*) 1, 2, 7; (*b*) 3, 4, 5, 6, 8; (*c*) 9, 12, 14, 15, 17; (*d*) 10, 11, 13, 20, 22, 24; (*e*) 16, 18, 19, 21, 28; (*f*) 23, 25, 27, 29, 31, 32. Monospecific antisera are, of course, required also. The pneumococcus to be typed is tested with each pool and then with the component antisera of the pool with which it reacts. Thus a pneumococcus may be identified in twelve or less tests.

The sputum may be used directly in the *Quellung* reaction if there are sufficient pneumococci present, or it may be heated and centrifuged and the supernatant used as antigen in a precipitin-ring test (Krumwiede's method). In the latter instance the reaction is dependent upon the pres-

[20] Methods of typing are discussed in detail on pp. 620–640 of Heffron: *Pneumonia.* The Commonwealth Fund. New York. 1939.

[21] Neufeld: Ztschr. f. Hyg. u. Infektionskr., 1902, *40*:54.

[22] Neufeld and Etinger-Tulczynska: Ztschr. f. Hyg. u. Infektionskr., 1931, *112*:492.

[23] Kempf and Nungester: Jour. Inf. Dis., 1942, *71*:50.

ence of SSS in the sputum, and a negative reaction has no significance because of the possible lack of sufficient SSS.

The intraperitoneal inoculation of white mice with sputum results in a rapid growth of pneumococci, and peritoneal exudate aspirated after three to six hours contains large numbers of bacteria as well as considerable amounts of SSS. The bacteria may be typed by the *Quellung* reaction, by microscopic slide agglutination (Sabin's method), or by macroscopic agglutination or precipitin tests. When mice are not available the sputum may be cultured in glucose blood broth (Avery's method) and the broth and contained pneumococci used as antigen in the immunological tests.

Whatever method or combination of methods is used, the time element is an important factor, for typing is a necessary preliminary to the intelligent administration of antiserum.

Variation.—The smooth and rough variants that have been found in a variety of bacteria may also be observed in the pneumococcus. As in other cases, there are various intermediate colonial types between the two extremes, and the pneumococcus is virulent in the smooth form and almost completely avirulent in the rough form. The change from smooth to rough is reflected in the microscopic morphology of the cells as a loss of capsule and the ability to form SSS. It was first observed by Stryker[24] that pneumococci cultivated in homologous immune serum did not form capsules; the ability of specific antibody to inhibit the elaboration of capsular substance and thereby render them susceptible to phagocytosis is no doubt an important consequence of the immune response. Since type-specificity is determined by the SSS, it follows that the change from smooth to rough is accompanied by a complete loss of type-specificity; the somatic antigen is predominant and, irrespective of original type, the pneumococci become immunologically identical. The dissociative change may be reversed, although with difficulty, by animal passage or by cultivating the R form in the presence of anti-R immune serum, or in the presence of heat killed cells from a smooth culture.

Transformation of Types.—Of fundamental biological significance, however, was the discovery of Griffith[25] that the inoculation of mice with living R culture mixed with a heat-killed suspension of smooth pneumococci of a type other than that from which the R culture was derived resulted in the conversion of the R variant to the S variety of the new type. These transformations of pneumococcus type have also been brought about *in vitro* by Dawson and Sia,[26] by cultivation of R variants in blood broth in the presence of anti-R immune serum and heat-killed smooth pneumococci of heterologous type. More recent work on these transformations and their significance to bacterial variation is discussed earlier (p. 156). The S-R variation occurs in nature (its practical importance to human pneumococcus infections is not clear) and R variants may be isolated from pneumococcus infections in man,[27] but whether the transformation of types likewise occurs in nature is not known.

[24] Stryker: Jour. Exp. Med., 1916, *24*:49.
[25] Griffith: Jour. Hyg., 1928, *27*:113.
[26] Dawson and Sia: Jour. Exp. Med., 1931, *54*:681.
[27] Shibley and Rogers: Proc. Soc. Exp. Biol. Med., 1932, *30*:6.

Pathogenicity for Man.—As indicated above, lobar pneumonia is the most important pneumococcal infection in man. The bacteria are not confined to the lung, however, for they may migrate from this seat of infection through the nasal passages or be distributed via the vascular system to various parts of the body, to give rise to localized foci of infection. Pneumococcemia is of frequent occurrence; available data indicate that 50 per cent is a fair estimate. A number of workers emphasize the prognostic value of blood culture, and in most instances the case fatality is considerably higher when pneumococci are present in the blood stream. Avery, Chickering, Cole and Dochez[28] observed a case fatality of 55.8 per cent in those with positive blood cultures and 8.3 per cent of patients with negative culture. Similar results have been reported by others.[29]

Among the pathologic processes that occur as complications and sequelae of pneumococcus pneumonia, or, it may be noted, as independent and primary affections, are inflammations of the pleura, pericardium and meninges. Meningitis and otitis media are frequently secondary to pneumonia, and the connection between inflammation of the middle ear and meningeal infection has often been noted. To those inflammatory conditions of pneumococcal origin might be added a long list of others provoked by the same organism. There appear to be few, if any, organs or tissues that are not under some circumstances subject to attack. Sinusitis, parotitis, conjunctivitis, enteritis and a great variety of other affections are occasionally due to the activity of pneumococci. In general, pneumococcus infections of this type tend to a more favorable outcome than similar infections with streptococci or staphylococci.

Resistance to pneumococcal infection is, in large part, a matter of individual predisposition, and the mere presence of pneumococci in the upper respiratory tract is not alone sufficient to bring about pulmonary infection. Specific immunity plays a negligible part in resistance, but the complex of factors associated with a state of physiological well-being is of the greatest significance. A preliminary depression of resistance by other infections, severe or sudden exposure to cold, fatigue and other predisposing factors, is an almost invariable preliminary to pneumococcus infection. The part played by pneumococcus pneumonia in the fatal termination of many diseases, for example, is well known.

The Pathogenicity of Pneumococcus Types.—The case fatality of pneumococcus pneumonia is relatively high and indicative of the pathogenicity of these bacteria once they have become established in the lungs. The pneumococcus types differ from one another in this respect; the case fatality in Type I infections is 25 to 30 per cent, that of Type II about 40 per cent, that of Type III 40 to 60 per cent, and that of Group IV infections perhaps 15 to 20 per cent. Because of the relatively recent differentiation of the types comprising Group IV, data on case-fatality rates for these types are very meager as yet. The frequency of occurrence of the pneumococcal types in lobar pneumonia and bronchopneumonia is given in Fig. 50. Here the

[28] Avery, Chickering, Cole and Dochez: *Acute Lobar Pneumonia.* Monographs of the Rockefeller Institute for Medical Research No. 7, 1917.

[29] *Cf.* Christie: Lancet, 1932, *ii:*1173.

ten leading types are: I, II, III, IV, V, VI, VII, VIII, XIV and XIX. In some series Type II is more frequent than Type III and in others, including this one, the reverse is true.

Pneumococcus Carriers.—As a strict parasite, the pneumococcus is found in man rather than in his environment. Healthy carriers of these bacteria are common; 40 to 60 per cent of groups of persons examined have been found to harbor pneumococci in the upper respiratory tract. This proportion is variable, however, being relatively high during the cold months of the year and higher among groups of contacts than among non-contacts. The carrier state is not permanent but rather sporadic and intermittent; many persons may carry these bacteria for a short time, particularly while

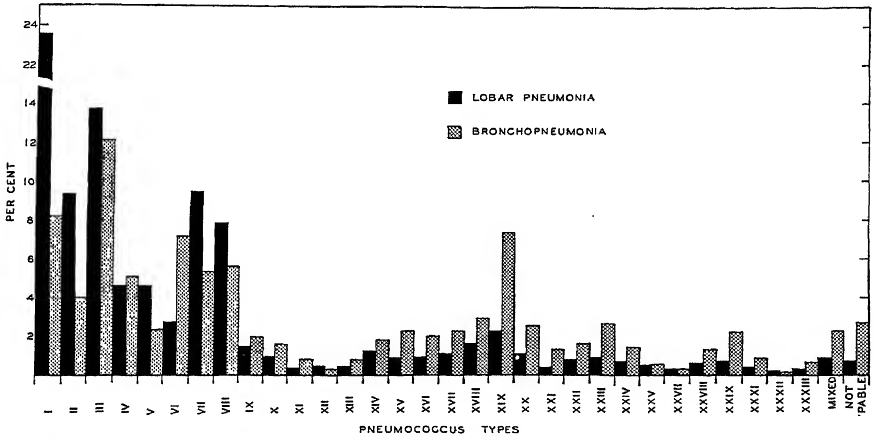


Fig. 50.—The incidence of pneumococcus types in lobar and bronchopneumonia observed in a two-year survey in California, Colorado, Illinois, Louisiana, New Jersey and Missouri in which pneumococci from 12,447 cases of lobar pneumonia and 3,847 of bronchopneumonia were typed. The relative predominance of Type III over Type II is sometimes observed. Note the more common occurrence of higher types in bronchopneumonia, the relative incidence of mixed types, *i.e.*, those reacting to more than one antiserum, and the incidence of types other than the first thirty-three. Data from Rumreich *et al.*: Pub. Health Repts., 1943, *58*:121.

having colds and other infections of the upper respiratory tract, while others may carry them for longer periods. There is also a seasonal fluctuation in that increased numbers are found in the winter months.

The great majority of pneumococci found in carriers are the relatively less virulent types of Group IV. Types I, II and III are much less frequently found, but an appreciable portion of the population may harbor these types at any one time (see table). In the study made by Smillie, Calderone and Onslow[30] almost every type of pneumococcus was encountered; many individuals carried two or more types simultaneously. The types most commonly found were III, VII, XXI, XXV and XI in that order of fre-

[30] Smillie, Calderone and Onslow: Amer. Jour. Hyg., 1943, *37*:156.

quency. It is not clear whether an appreciably effective immunity is developed by carriers.[31]

The Epidemiology of Pneumococcus Pneumonia.[32]—Pneumococci are disseminated chiefly through the secretions and discharges of the mouth and upper respiratory tract by direct contact between persons. Droplet infection (p. 201) undoubtedly plays a large part in the transmission of these microorganisms and perhaps accounts for the seasonal incidence of the disease (Fig. 51) and the increased frequency of carriers during the cold months of the year.

While it is obvious that pneumococcus infection is always exogenous in the last analysis, for practical purposes it is probably endogenous in a large proportion of cases. With a high carrier rate pneumococci are frequently present in the normal individual, and when resistance is reduced to a sufficiently low level they are able to set up an infection. The high incidence of pneumococci of Types I and II (Fig. 50) as contrasted with the low

PNEUMOCOCCUS CARRIERS*

Persons Examined				Incidence of Carriers				
	Total	Cases Found		Total†	Per Cent Incidence of Types			
		Number	Per Cent		I	II	III	Group IV
Non-contacts.....	2332	1000	42.9	1027	0.5	0.9	8.4	34.2
Contacts.........	1782	977	54.8	1018	3.3	2.7	10.0	41.0

* Modified from Heffron's[20] data.
† The incidence of types is greater than the incidence of carriers because in some instances more than one type was found.

carrier incidence of these types, together with the occurrence of seeming epidemics of pneumococcus pneumonia on a small scale, has been regarded by some as evidence of exogenous infection. It is more probable, however, that the greater virulence of some pneumococcus types operates as a selective factor to disturb the random distribution of types in pneumococcus pneumonia. It is known, for example, that minor respiratory infections may be common or epidemic in small groups such as a family. If a given type of pneumococcus of high virulence invades and spreads within the group so that a high proportion of the individuals become carriers, the operation of factors which reduce resistance in the group may result in one or more members coming down with pneumonia due to the type carried. Smillie and Jewett[33] observed just such a sequence of events in a group of children in a nursery. The group was invaded by a virulent Type XIV pneumococcus which caused no harm but when the individual developed an acute respiratory infection, the dormant pneumococcus spread to the middle ear, con-

[31] *Cf.* Finland, Brown and Barnes: Amer. Jour. Hyg., Sec. B, 1940, *32*:24.
[32] See the review by Finland: Medicine, 1942, *21*:307.

junctivae and lungs. Such children were sent to the hospital ward and carried the pneumococcus which spread to most of the children there. Again, the infection was activated, sometimes with very serious consequences, on the development of respiratory infection.

Other epidemiological characteristics of pneumococcus pneumonia include seasonal incidence (Fig. 51), which corresponds roughly with the carrier rate; the age incidence, characterized by high morbidity and mortality in infants and the aged; the higher incidence in males than in females; and the apparent greater susceptibility of the Negro as contrasted with the white race.

Bacteriological Diagnosis of Pneumococcus Infections.—The pneumococcus may be isolated by culture or animal inoculation from specimens such as sputum, pleural exudate, blood, spinal fluid, pus, etc. Blood agar is the medium of choice, the bacteria growing up in twenty-four hours as

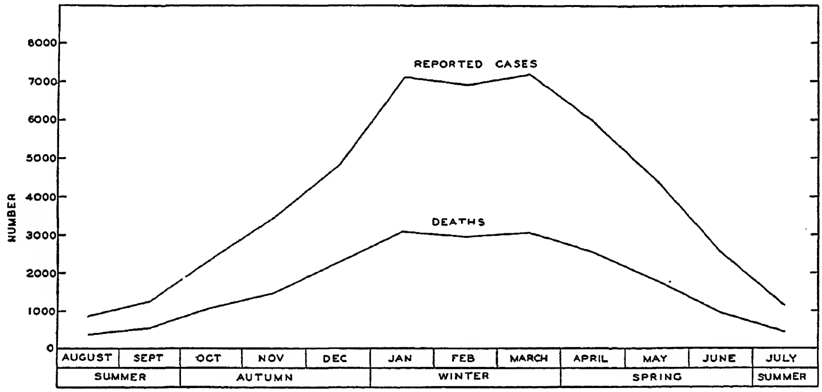


Fig. 51.—The seasonal incidence of pneumococcus pneumonia as indicated by the monthly distribution of 47,947 reported cases and 20,544 reported deaths in Massachusetts, 1921–30. Data from Heffron.[20]

small colonies surrounded by a zone of green hemolysis. It is not possible to distinguish them from α-hemolytic streptococci by colonial or microscopic morphology but differentiation may be made by the fermentation of inulin and bile solubility of the pneumococcus and its immunological reactions. Blood specimens are cultured in buffered dextrose veal infusion broth, containing 5 mg. per 100 cc. of *p*-aminobenzoic acid if the individual is undergoing sulfa drug therapy, as in the case of culture of the streptococci. A portion of sputum, washed in three changes of sterile saline and emulsified in saline, may be inoculated intraperitoneally in a white mouse. With virulent strains the animal will show signs of illness in five to eight hours and microscopic examination of smears of peritoneal exudate will show large numbers of encapsulated diplococci.

The pneumococcus is identified and typed with antiserum, usually by the *Quellung* reaction through agglutination; precipitin tests may be used

[33] Smillie and Jewett: Amer. Jour. Pub. Health., 1942, *32*:987.

also. When large numbers of the bacteria are present in sputum, the typing may be done directly without culture or mouse inoculation, but since the *Quellung* reaction is inhibited in the presence of large amounts of SSS, negative reactions are not significant. Typing is readily carried out, as indicated earlier, on pneumococci present in mouse peritoneal exudate or cultures.

Pathogenicity for Lower Animals.—The susceptibility of the usual laboratory animals to pneumococcus infection is variable, ranging from the highly susceptible mouse and rabbit through the less sensitive guinea pig to the cat, dog, chicken and pigeon, which are highly resistant. Animal experiments with the pneumococcus present an example of the general law that susceptibility is characterized by general septicemic infection, resistance by the occurrence of a localized process. The mouse and the rabbit develop a rapidly fatal septicemia, and in these animals lung lesions, when they occur at all, are slight and usually limited to the bronchopneumonic type. It is possible to produce typical lobar pneumonia in the rabbit by carefully balancing the susceptibility of the animal and the virulence of the bacterium through the use of attenuated cultures or previous partial immunization. Resistant animals, such as the dog, show an approximation toward the type of pneumococcus infection observed in man, and lobar pneumonia may be produced in monkeys by intratracheal inoculation.[34] The lesions produced in monkeys were considered identical with those in human lobar pneumonia. It is of some interest that pneumococci were found in the blood within six hours after their introduction into the trachea and before the signs of pneumonia appeared, suggesting the bronchogenic rather than the hematogenous origin of the infection.

Man, therefore, may be regarded as an animal of rather high normal resistance. This resistance may, however, be so reduced as to permit the production of localized manifestations, which in still more susceptible individuals may lead to a fatal septicemia. In some cases death is due to overwhelming interference with respiration caused by the local pulmonary lesions; in others, to a general systemic poisoning or toxemia.

Immunity.—Experimental animals may be actively immunized against pneumococcus infection by the injection of vaccines of the smooth, virulent bacteria, although the immunity is not of long duration, *i.e.*, not more than a few months. The development of the immune state is accompanied by the appearance of antibodies, precipitins, agglutinins and the like, as well as a protective quality in the blood serum. In man the situation is somewhat obscure; there is undoubtedly an intimate relation between recovery and the appearance of humoral antibodies, but immunity following infection is slight and transitory, and one attack may succeed another after a short interval. Active immunity in experimental animals is type-specific, however, and it is not improbable that under natural conditions different immunological types may participate in successive attacks.

The results of studies on the active immunization of man have not been clear-cut. Although morbidity is high in the aggregate, it is not confined to particular age groups or environmental conditions and, in consequence, such studies are difficult. In some instances, however, suggestive results

[34] Blake and Cecil: Jour. Exp. Med., 1920, *31*:403.

have been obtained; mass inoculations carried out in the United States in 1918–19, for example, provided such evidence.[35] Of these studies the work of Gundel and Homann[36] has yielded promising results and Felton and his co-workers[37] have obtained suggestive results in extensive studies. The efficacy of the active immunization of man is, however, by no means established as yet.

The results of the therapeutic use of antiserum are variable, being excellent with some types of pneumococci and not with others. The treatment of Type I infections with specific antiserum is relatively successful in that the case fatality is reduced from 25 to 30 per cent to 12 per cent. Type II infections do not respond quite so well to serum therapy, the case fatality being reduced from about 40 per cent to 25 per cent, and Type III infections are little benefited. Of the types comprising Group IV, Types V and VII appear, on the basis of present evidence, to respond to serum therapy. Specific serum therapy is complicated by the existence of the multiplicity of higher types of pneumococci. Antisera have been available for Types IV, V, VII and XIV but it seems probable that polyvalent sera, based on the frequency of occurrence of the higher types, will become a practical necessity.[38] Combinations to give such polyvalent antisera have been suggested by Eddy.[39]

Antiserum is generally prepared in horses, but recent work[40] has suggested that rabbit antisera may be more effective, in part because higher protective titers are obtainable than with horse serum and in part because the smaller antibody molecules (p. 277) should facilitate absorption.

The standardization of antipneumococcus sera may be carried out either by the titration of antibody content in the test tube, *viz.*, agglutinins and precipitins, or by measurement of the protective qualities of the serum for experimentally infected mice.

It may be noted here that a bacterial enzyme decomposing the Type III specific polysaccharide isolated by Avery and Dubos[41] protects experimental animals against Type III pneumococcus infection.[42] The injection of the enzyme preparations, however, produces untoward reactions, notably a febrile reaction and leucopenia, and therefore they have not been used in man.

[35] Cecil: Medicine, 1925, *4*:395.

[36] Gundel and Homann: Deut. med. Wchnschr., 1938, *64*:917.

[37] *Cf.* Felton *et al.*: Pub. Health Repts., 1941, *56*:1041 *et ante.*

[38] See the comprehensive review of this matter by Finland: Jour. Amer. Med. Assn., 1942, *120*:1294.

[39] Eddy: Pub. Health Repts., 1944, *59*:1485.

[40] Horsfall, Goodner and MacLeod: Science, 1936, *84*:579; Jour. Amer. Med. Assn., 1937, *108*:1483; Goodner, Horsfall and Dubos: Jour. Immunol., 1937, *33*:279.

[41] Avery and Dubos: Science, 1930, *72*:151.

[42] Avery and Dubos: Jour. Exp. Med., 1931, *54*:73; Goodner, Dubos and Avery Jour. Exp. Med., 1932, *55*:393.

CHAPTER 17

THE GRAM-NEGATIVE PATHOGENIC COCCI (NEISSERIA): THE GONOCOCCUS AND THE MENINGOCOCCUS

Family: *Neisseriaceae*. Genus: *Neisseria*. Strict parasites, failing to grow or growing very poorly on ordinary artificial media, growth fairly abundant on serum media. Gram-negative cocci, usually in pairs. Aerobic. Type species: *Neisseria gonorrheae*.

The gonococcus and the meningococcus are the chief representatives of a small group of closely related bacteria whose other members are non-pathogenic inhabitants of the mouth and upper respiratory tract of man. Two groups of species are separated from one another on the basis of pigment production, and further differentiation is made by means of fermentation reactions:

FERMENTATION REACTIONS OF THE GRAM-NEGATIVE DIPLOCOCCI

Non-pigmented Species	Dextrose	Maltose	Sucrose	Levulose	Mannitol
N. gonorrheae (gonococcus)	+	−	−	−	−
N. intracellularis (meningococcus)	+	+	−	−	−
N. catarrhalis	−	−	−	−	−
N. sicca	+	+	+	+	−

Pigmented varieties are frequently found in the nasopharynx (*Neisseria flava* I, II, III).

THE GONOCOCCUS[1]

Neisser[2] in 1879 first called attention to the constant presence of a peculiar coccus in gonorrheal pus. In cases of gonorrhea of recent origin this was the sole organism found; it not only occurred in the urethral and vaginal discharges of ordinary gonorrhea, but was present in the exudate in conjunctivitis due to gonorrheal infection. Pure cultures of this microorganism were isolated by Bumm[3] in 1885, who succeeded in demonstrating its etiologic relation to gonorrhea by the inoculation of human volunteers. This bacterium, known generally as the *gonococcus*, has been termed *Micrococcus gonorrheae* and *Diplococcus gonorrheae*, but the genus *Neisseria* is

[1] Present knowledge of gonorrhea is reviewed in the report of the Committee for Survey of Research on the Gonococcus and Gonococcal Infection. Thomas and Bayne-Jones: Amer. Jour. Syph., 1936, *20*:Suppl. to No. 1.

[2] Neisser: Centralbl. f. d. med. Wissensch., 1879, *17*:497.

[3] Bumm: *Der Mikroorganismus der gonorrhoischen Schleimhauterkrankungen*. Wiesbaden, 1885.

now more or less generally accepted and this bacterium is properly known as *Neisseria gonorrheae.*

Morphology and Staining.—In preparations made from gonorrheal pus the cells of the gonococcus occur in pairs, with the flattened sides in juxtaposition; the appearance in stained preparations resembles that of a coffee bean. In pure culture the cocci appear as oval or spherical and are often aggregated in irregular masses without the typical diplococcus arrangement. In pus smears the gonococcus occurs almost entirely within the leucocytes; frequently enormous numbers may be found packed within a single phagocyte. In the earliest stages of infection, however, gonococci may be found extracellularly, and the same is true of cases of gonorrhea of long standing. The gonococcus is nonmotile and does not form spores.

The colonies of the gonococcus are small, translucent, finely granular with lobate margin and grayish white with a pearly opalescence when

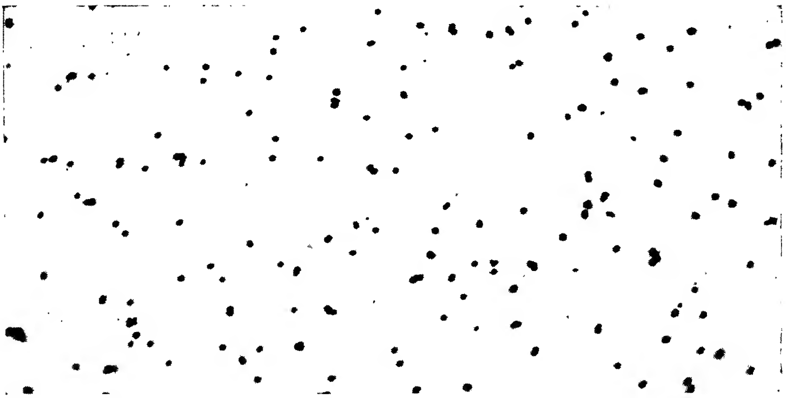


Fig. 52.—The gonococcus from pure culture. Fuchsin; × 1050.

viewed by transmitted light. Larger colonies may be formed on special media. Colonial appearance is, however, subject to considerable variation (see below).

Unlike the pyogenic cocci, the gonococcus and related forms are gram-negative, a staining characteristic that is of considerable diagnostic value since it serves to differentiate the gonococcus from other cocci present in the urethral or vulvovaginal tracts. Other gram-negative cocci may be found occasionally, sometimes within the leucocytes, but they are rare. The tendency to decolorization in the gram stain is variable. Some strains decolorize much more readily than others, and gonococci embedded in masses of pus may retain the stain; hence the preparation of thin, uniform films is highly desirable. The gonococcus stains with the aniline dyes, but polychrome stains, such as Pappenheim's stain,[4] are more useful. Intra-cellular granules may be found in stained preparations, but in general the

[4] A methyl green-pyronine stain.

gonococci from young cultures stain evenly, while older cultures (twenty-four hours and older) contain large swollen involution forms which may stain poorly.

Physiology.—In its nutritive requirements the gonococcus is one of the most fastidious bacteria, particularly upon primary isolation. An enriched medium is, of course, required for cultivation. Earlier media were enriched by the addition of ascitic and hydrocele fluid. A proteose-3 hemoglobin agar has been used to some extent in this country, but the most satisfactory medium is chocolate (heated blood) agar prepared with an infusion base. Nutritional requirements appear to be highly complex and are not well known. Mueller and Hinton[5] have devised a protein-free hydrolyzed casein-starch medium that may be autoclaved and Boor[6] has been able to grow the gonococcus on a medium containing tryptic digest of casein, cystine, dextrose and buffer. The cystine appears to be required by the gonococcus but not by the meningococcus. A sufficient supply of moisture is essential, there should be water of condensation on the tubes or plates and the atmosphere of the incubator should be kept saturated with water. Incubation in an atmosphere of increased CO_2 tension, about 10 per cent, greatly improves growth and is a practical necessity in primary isolation.[7] With continued cultivation on laboratory media, the gonococcus appears to become somewhat less fastidious and some strains may eventually grow upon the ordinary infusion media. The preservation of cultures is difficult, however, for the gonococci die off in two to three days at room temperature and in six to eight days at 37° C. but will live longer when kept in the cold. Even upon continued transfer the gonococci die off and cultures are frequently lost. The optimum temperature for growth is 37° C., growth does not occur below 30° C., and temperatures of 40° to 41° C. are definitely harmful. The gonococcus will grow sparsely under anaerobic conditions but is essentially aerobic in character.

With respect to deleterious influences the gonococcus is a delicate micro-organism. It is readily killed by heat as indicated above and by dilute antiseptics; 1 per cent phenol, for example, kills in one to three minutes. It is remarkably sensitive to certain of the flavine dyes (p. 69) and is rapidly destroyed by silver salts. The gonococcus is sensitive to drying and, under ordinary conditions, can survive exposure to the air for only a very short time—one to two hours—although in masses of dried pus it may live exceptionally for six to seven weeks.

The gonococcus is not very active biochemically. Glucose is fermented, principally to lactic acid, but many other sugars are not attacked, indol is not produced, nitrates are not reduced, and no change is produced in litmus milk. Catalase is produced, and a characteristic that has been turned to practical differential use is the formation of indophenol oxidase. McLeod *et al.*[7] recommend the cultivation of suspected material on 10 per cent

[5] Mueller and Hinton: Proc. Soc. Exp. Biol. Med., 1941, *48:*330.

[6] Boor: Proc. Soc. Exp. Biol. Med., 1942, *50:*22.

[7] McLeod, Coates, Happold, Priestly and Wheatley: Jour. Path. Bact., 1934, *39:*221. See also Thompson: Jour. Inf. Dis., 1937, *61:*129; Malcolm and Dolman: Canadian Pub. Health Jour., 1939, *30:*252.

heated blood (chocolate) agar in an atmosphere containing 8 per cent carbon dioxide, followed by twenty-four hours' incubation in air. A 1 per cent solution of tetramethyl-*p*-phenylenediamine is poured on the incubated plate and poured off again immediately or sprayed on with a nasal atomizer. Colonies of bacteria forming indophenol oxidase turn a bright purple color. The bacteria are not immediately killed and may be subcultured within half an hour. This so-called "oxidase reaction," coupled with the examination of smears for gram-negative intracellular diplococci, is becoming generally used in the laboratory diagnosis of gonorrhea.

Toxins.—Although a number of attempts were made by the early workers to demonstrate the formation of toxins by the gonococcus, variable results were obtained. These bacteria form a weak hemolysin, and their cell substance will kill laboratory animals when injected in sufficient amount and will evoke suppuration upon instillation into the human urethra. The

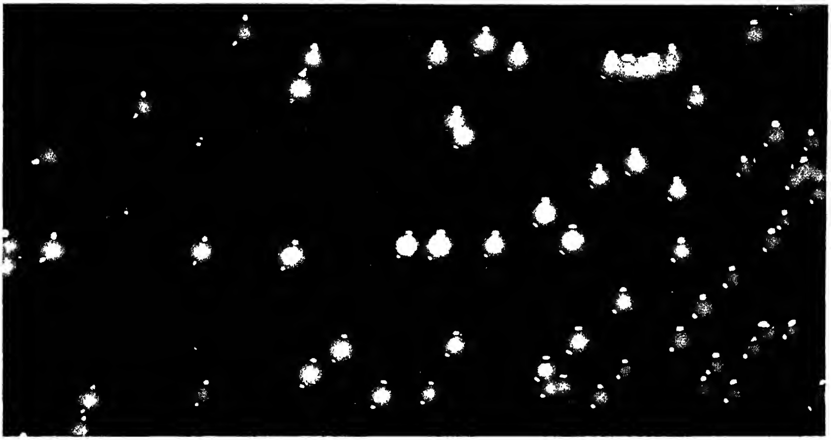


Fig. 53.—Colonies of the gonococcus on blood agar. × 6.

products of other bacteria, such as staphylococci and colon bacilli, will produce the same result, however, and it may be concluded that the gonococcus does not produce a specific toxin.

Variation.—Numerous workers have observed that the cultural characteristics of the gonococcus are subject to considerable variation. More recently it has been found[8] that two types of colonies may be observed which appear to be correlated with immunologic type. The one, designated as Type I, is a large, irregular, flattened, translucent colony that, on continued incubation, gives rise to surface papillae; the other type of colony, Type II, is somewhat smaller, round, raised with slightly convex uneven surface, and opaque. Type I colonies are generally recovered from acute gonorrhea, while Type II are found in old laboratory strains and, sometimes, in chronic gonorrhea. The papillae are thought to represent the first

[8] Atkin: Brit. Jour. Exp. Path., 1925, *6*:235.

step in transition toward the Type II form. The relation of these forms to the dissociative changes of other bacteria is uncertain.

Classification.—The relationship of the gonococcus to the other members of its genus has already been discussed and need not be considered further. The gonococci are immunologically heterogeneous, and a number of attempts have been made to subdivide them into types. Most of the freshly isolated strains from acute cases appear to fall in one serological group, while old stock cultures and strains from chronic cases constitute a second subdivision[9] which is regarded by some as a degenerative form.[10] "Intermediate" and serologically "independent" strains occur, and a sharp differentiation may not be made. Recent work[11] on the chemical fractionation of gonococci has indicated the presence of polysaccharide and nucleoprotein components which are shared by other species of *Neisseria*, the meningococcus and *Micrococcus catarrhalis*. Type-specific carbohydrate has been found in gonococci which is said to be responsible for the immunological character of Type I and Type II, the two groups noted above.[12] According to Phair, Smith and Root[13] high titer specific agglutinating antisera may be prepared in chickens by the intravenous inoculation of living gonococci. The significance of these findings to gonococcal infection is as yet uncertain.

Pathogenicity for Man.—Few diseases are so widely disseminated through all classes of society as gonorrhea. Precise information as to the incidence of the disease is not available; a morbidity rate of 10 per cent in the United States is generally accepted as a conservative estimate. It is calculated that about a million fresh gonococcal infections occur each year, and there is no evidence of a downward trend.[14]

As a rule the gonococcus attacks primarily the human urethra and gives rise to an inflammation which may be followed by chronic urethritis and stricture. There is a marked tendency for spread of the infection along contiguous mucous surfaces, resulting, in the male, in epididymitis and other inflammatory conditions. In the female, the entire genito-urinary tract may be involved, and the fallopian tubes, the ovaries and the peritoneum are not uncommonly invaded. The gonococcus may also invade the blood stream from local lesions and be carried to various parts of the body and give rise to a variety of extragenital lesions. Especial predilection is shown for the synovial membranes of the joints, where it causes the so-called "gonorrheal rheumatism," and for the heart valves, where it produces endocarditis. Local or general complications occur in perhaps 30 per cent of all cases. Gonococcal meningitis occurs, perhaps more frequently than formerly thought.[15]

Once established, gonococcal infection persists for a long time; five to

[9] *Cf.* Casper: Jour. Bact., 1937, *34*:353.

[10] *Cf.* Casper: Jour. Bact., 1938, *36*:111.

[11] Boor and Miller: Jour. Exp. Med., 1934, *59*:63; Miller and Boor: Jour. Exp. Med., 1934, *59*:75.

[12] Casper: Jour. Immunol., 1937, *32*:421.

[13] Phair, Smith and Root: Proc. Soc. Exp. Biol. Med., 1943, *52*:72.

[14] Vonderlehr and Usilton: Amer. Jour. Syph., 1938, *22*:537.

[15] Branham, Mitchell and Brainin: Jour. Amer. Med. Assn., 1938, *110*:1804.

fifteen years' duration has been reported but exclusion of reinfection is a difficult matter. Carpenter and Westphal,[16] however, have observed infection of seven years' duration without reinfection. In general, very little is as yet known of the gonococcus carrier or the part played by the carrier in the spread of the disease.

Gonorrheal vulvovaginitis occurs in epidemic form in little girls, and in these instances the infection is transmitted by bedclothes, towels, common bathtubs and other fomites. The commonest complications are urethritis, proctitis and cervicitis. Such epidemics are frequently exceedingly difficult to control and constitute a serious problem in many institutions such as children's wards in hospitals.[17]

Gonorrheal ophthalmia of the newborn is a well-known consequence of maternal infection; infection does not occur *in utero* but during passage through the birth canal. Although exact information is not obtainable, it is

Fig. 54.—Urethral smear from gonorrhea. Gram stain. Note the intra- and extracellular position of the gonococci and their typical coffee-bean shape and arrangement in pairs. × 2400.

estimated that 10 per cent of all cases of blindness are traceable to this source, and that in the United States there are perhaps 12,000 children blind from this cause. The instillation of silver nitrate or other silver salts immediately after birth prevents infection.

Except in the case of vulvovaginitis in children, gonorrhea is spread by direct contact, usually sexual. Once infected, an individual may remain infective for a long time, and gonococci may persist in the genito-urinary secretions for years after apparently complete recovery, and even though they may not be found by bacteriological examination the infection may be transmitted. Gonorrhea persists, then, in the human population in a smol-

[16] Carpenter and Westphal: Amer. Jour. Pub. Health, 1940, *30*:537.

[17] A comprehensive discussion of vulvovaginitis in children may be found in the Medical Officer, 1938, *59*:191, 203; see also Cohn, Steer and Adler: Ven. Dis. Inform., 1940, *21*:208.

dering endemic form and, as a consequence of the nature of its transmission, widespread epidemics in the usual sense do not occur.

Bacteriological Diagnosis of Gonococcus Infection.[18]—The demonstration and identification of the gonococcus are essential to the diagnosis of gonorrhea and it should be borne in mind that every case of gonorrhea is potentially a medico-legal case. The presence of gram-negative intracellular diplococci in urethral smears in most cases justifies a provisional diagnosis of gonorrhea. Morphologically similar bacteria may, however, be present in the vagina or conjunctivae.[19] It is generally agreed that culture of the gonococcus gives a higher proportion of positive results than direct smear examination alone[20] and cultured gonococci may, of course, be identified. The question of survival of this relatively delicate bacterium in transport of specimens is of some importance. It seems to survive well in urine[21] and

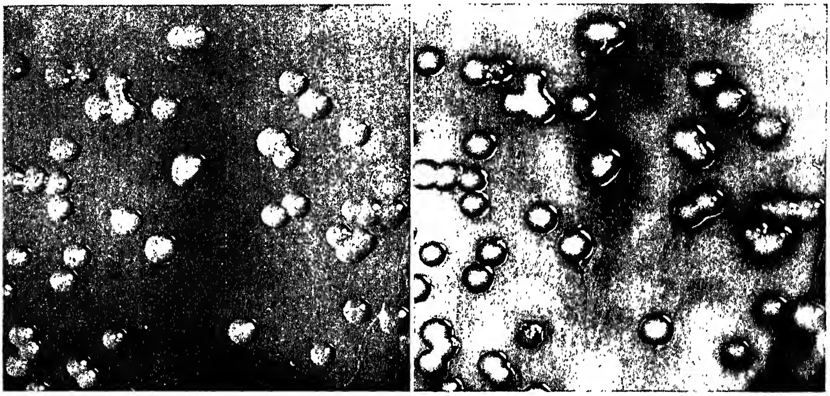


Fig. 55.—The oxidase test for the identification of gonococcus colonies. Pure culture on blood agar. Left, gonococcus colonies before the application of tetramethyl-*p*-phenylenediamine solution. Right, the same colonies after the application of the reagent. Note the greater intensity of color about the edges of the colonies immediately after application, and the discoloration of the medium. × 5.

Cox, McDermott and Mueller[22] have devised a diluted defibrinated blood solution containing 1:30,000 crystal violet into which the swab is placed; the dye serves to inhibit the growth of contaminants. For culture infusion base-chocolate agar (often designated "for *Neisseria*") is the medium of choice and is inoculated directly with the specimen (or sediment if it is urine or spinal fluid). The culture must be incubated in 10 per cent CO_2; this atmosphere may be satisfactorily approximated by putting the plates

[18] For a recent and detailed summary of the laboratory procedures see Carpenter: Ven. Dis. Inform., 1943, *24:*133.

[19] *Cf.* De Bord: Jour. Lab. Clin. Med., 1943, *28:*710.

[20] For example, see Sulkin and Gottlieb: Amer. Jour. Syph., 1941, *25:*22; Weiss and Colvin: Southern Med. Jour., 1941, *34:*1102.

[21] Allison, Charles and Carpenter: Ven. Dis. Inform., 1942, *23:*283.

[22] Cox, McDermott and Mueller: Ven. Dis. Inform., 1942, *23:*226; Amer. Jour. Pub. Health, 1943, *33:*149.

in a jar together with a lighted candle and sealing. The oxidase test serves to differentiate the oxidase-positive colonies and, if picked immediately, they may be subcultured. Identification is based upon sugar fermentations in serum broth.

Pathogenicity for Lower Animals.—The gonococcus is non-pathogenic for lower animals, aside from the toxicity of the cell substance as noted above, and gonorrhea has never been reproduced in experimental animals, including anthropoid apes.

Immunity.—Little if any immunity to the gonococcus is acquired as a result of infection, and second and third infections may be superimposed upon the first, *i.e.*, acute upon old chronic infections. As might be expected, then, the therapeutic use of vaccines and various types of antisera is without effect. The significance of the observed extensive phagocytosis of gonococci by polymorphonuclear leucocytes is uncertain.

Some degree of immunological response is evident, however. Complement-fixing antibodies are usually present, and patients may give a marked skin reaction to suspensions of killed gonococci. A number of attempts have been made to utilize these responses in the immunological diagnosis of gonorrhea.[23] The complement-fixation test has shown some promise but is not generally used. The skin reaction is apparently too variable to have practical value.[24] It has also been observed that the discharges from gonorrheal inflammations give a precipitin reaction with antigonococcus serum, but this flocculation reaction has as yet no diagnostic value.

THE MENINGOCOCCUS[25]

Inflammation of the meninges or investing membranes (pia-arachnoid) of the brain and spinal cord may be provoked by a variety of microorganisms, and may occur either as a primary affection or secondarily in the train of an infection originally begun elsewhere. One form of meningitis, characterized especially by epidemic spread and usually designated as *epidemic cerebrospinal meningitis*, spotted fever or cerebrospinal fever, is accompanied by a specific microorganism commonly known as the meningococcus.

This bacterium was described by Marchiafava and Celli in the meningeal exudate as early as 1884, but the first important work upon it was that of Weichselbaum,[26] who, in 1887, obtained it in pure culture and described it in detail as the characteristic micrococcus found in six cases of acute cerebrospinal meningitis. Confirmation was supplied by the work of Jäger[27] in spite of some faulty observation. The etiologic rôle of the meningococcus has since been securely established by a number of investigations.[28]

[23] *Cf.* Casper: Ven. Dis. Inform., 1941, *22:*119.
[24] *Cf.* Torrey: Jour. Immunol., 1940, *38:*413.
[25] For general reviews see Murray: Med. Res. Council Spec. Rept., Ser. No. 124, 1929; Branham: Bact. Rev., 1940, *4:*59.
[26] Weichselbaum: Fortschr. d. Med., 1887, *5:*573.
[27] Jäger: Ztschr. f. Hyg., 1895, *19:*351.
[28] *Cf.* Foster and Gaskell: *Cerebrospinal Fever.* Cambridge University Press. London. 1916.

The meningococcus has been designated by a variety of names, including *Micrococcus meningitidis*, *Micrococcus intracellularis meningitidis*, *Neisseria meningitidis* and, according to Bergey,[29] *Neisseria intracellularis*. Although *Neisseria* is generally accepted, common usage is divided between *intracellularis* and *meningitidis*.

Morphology and Staining.—In film preparations of the meningeal exudate the meningococcus is very like the gonococcus and occurs in pairs or tetrads both within the leucocytes and free. The diplococci are flattened toward one another like gonococci, and there is considerable variability in the size of different cells in the same smear. In cultures the meningococcus averages a little less than 1 μ in diameter, and appears, as a rule, in pairs; short chains are seen more rarely. The variability in size observed in meningeal exudate may also be seen in cultures, particularly in those more than twenty-four hours old. Involution forms are common, and it is not unlikely

Fig. 56.—Meningococcus, pure culture. Note the typical diplococcus arrangement. Fuchsin; × 1050.

that the larger cells are degenerative. No capsule is present, although irregularities in staining and the occurrence of swollen cells have led to some confusion on this point. The meningococcus is non-motile and does not form spores.

Meningococcus colonies in blood agar are moist, elevated, smooth and with a bluish gray tinge. They do not produce green discoloration nor hemolysis and may be readily differentiated from the hemolytic and viridans streptococci and the pneumococcus. The colonies are not so white and opaque as those of the staphylococci.

The meningococcus stains readily with the usual aniline dyes and, like the gonococcus, is gram-negative. The involution forms found in cultures tend to stain unevenly, of course, but even young cells may show the presence of metachromatic granules when stained by Löffler's alkaline

[29] Bergey: *Manual of Determinative Bacteriology*. Williams & Wilkins Company. Baltimore. 5th ed., 1939.

methylene blue and other stains, and to a greater extent than the gonococcus. It may be noted that no sure distinction between the meningococcus and the gonococcus can be made on morphological grounds, and the identification of gonococci in gonococcal meningitis is dependent upon culture and differential fermentations.

Physiology.—Strains of meningococci vary considerably in the ease with which they may be cultivated; some strains will grow, although sparsely, on nutrient and infusion media, but in general rich media containing serum or whole blood are required. Infusion base chocolate agar or blood agar are the most useful media. The hydrolyzed casein-starch medium of Mueller and Hinton[5] and Boor's[6] tryptic digest medium will support growth of this bacterium. According to Boor the meningococcus differs from the gonococcus in that it does not require the addition of cystine to his

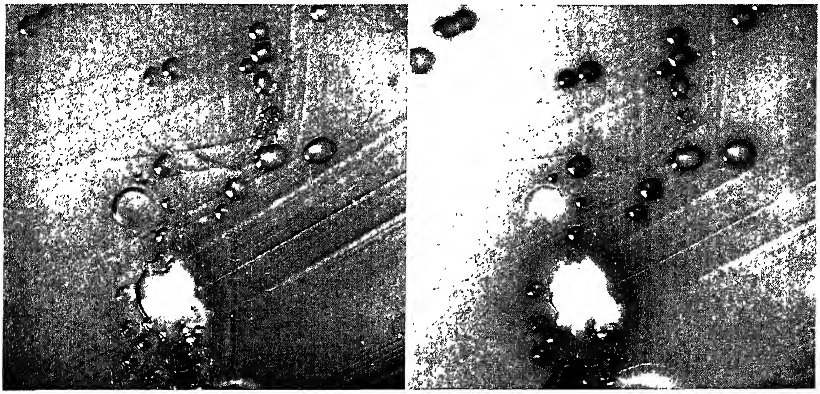


Fig. 57.—The oxidase test for the identification of meningococcus colonies. Mixed culture on blood agar. Left, colonies of meningococci and contaminants before the application of tetramethyl-*p*-phenylenediamine solution. Right, the same colonies after the application of the reagent. Note that the meningococcus colonies show the development of color first about the edges and there is slight discoloration of the medium. × 5.

medium. Frantz[30] has found that stock cultures of the meningococcus, which are, however, less exacting than recently isolated strains, will grow on an inorganic salt-glucose medium containing glutamic acid and cystine. In isolating the meningococcus it is essential that the culture medium be warm when inoculated and kept warm until finally placed in the incubator. Growth is favored, especially in primary isolation, by incubation in an atmosphere containing 10 per cent carbon dioxide. The meningococcus will grow over a temperature range of 25° to 42° C. with an optimum at 37° C. Although sparse growth will occur under anaerobic conditions, the meningococcus is, for all practical purposes, an aerobe.

Continued cultivation on laboratory media results in more luxuriant growth, and the bacteria presumably become less nutritionally fastidious. Meningococcus cultures are difficult to keep, however, and tend to die out

[30] Frantz: Jour. Bact., 1942, *43*:757.

in stock culture with refusal to grow in transplants. In most media these bacteria die within a few days if not transferred, but vitality may be preserved for several weeks in stab cultures in starch agar (1 per cent corn starch in nutrient agar) and are best kept in the incubator.

The relatively early appearance of involution forms in meningococcus cultures as well as their limited viability when not transplanted is, perhaps, attributable to their formation of an active autolysin, and in saline suspension in the incubator autolysis may take place within a few hours. The autolysin is heat-labile, being destroyed at 65° C. in thirty minutes, and suspensions prepared for agglutination studies should be inactivated in this way.

The meningococcus, like the gonococcus, is a delicate microorganism and not highly resistant to deleterious influences. It is killed in a short time by drying and by exposure to dilute disinfectants. It is particularly sensitive to heat and cold and, unlike many bacteria, dies out within a few days at 0° C.

The meningococcus is not an active fermenter. Considerable quantities of acid, presumably lactic for the most part, are formed from glucose and maltose. The fermentation of maltose serves to distinguish the meningococcus from the gonococcus. Neither is this bacterium actively proteolytic, for coagulated serum is not liquefied.

Toxins.—Meningococcus meningitis in man and that reproduced in experimental animals is usually accompanied by a profound toxemia. The meningococcus, however, appears to form no soluble toxin, though its cell substance is toxic to experimental animals when injected in relatively large amounts. Miller and his co-workers[31] have shown that the toxicity is heat stable (100° C. for thirty minutes) and the rate of its destruction suggests that the endotoxin consists of two substances, one much more thermostable than the other. The relationship of the endotoxin to the "P" substance (see below) is not clear.

Variation.—Rough and smooth colony types of the meningococcus have been described by Rake,[32] who found that recently isolated strains were generally smooth while old stock cultures were rough. Mucoid colonies were observed in a few instances. The change from smooth to rough was associated with a partial loss of immunological type-specificity.

Classification.—The meningococci are closely related to the gonococci, not only morphologically and physiologically but immunologically in that certain antigenic substances appear to be held in common as indicated previously.

The meningococci are not themselves immunologically homogeneous, and it was found by Dopter[33] that certain strains, culturally "typical" meningococci, did not agglutinate with antimeningococcus serum. These he designated "parameningococcus," a term that has since given rise to some confusion and may well be discarded. Gordon and Murray[34] distinguished four

[31] Miller *et al.:* Jour. Inf. Dis., 1943, *73*:248.
[32] Rake: Jour. Exp. Med., 1933, *57*:549, 561.
[33] Dopter: Compt. rend. Soc. biol., 1919, *66*:1055.
[34] Gordon and Murray: Jour. Roy. Army Med. Corps, 1915, *25*:411.

serological types, which they disignated by Roman numerals. These types have been telescoped into two groups, Group I containing Types I and III which are very closely related, and Group II made up of Types II and IV. Type IV seems to have disappeared (it is doubtful whether the European Type IV and American Type IV were identical) in recent years and Group II and Type II are synonymous. There is considerable variation in the frequency of occurrence of the two groups, and in general Group I appears to be associated with the epidemic disease while Group II predominates in interepidemic years. Recently a new type, related to Type II but independent and homogeneous, has been found to be quite prevalent.[35] It has been designated Type IIα. The types of meningococci encountered at present are, then, Group I, Group (or Type) II. and Type IIα.

While it is not uncommon to encounter strains of meningococci which do not fall into these types, most strains may be assigned to one or another of them. In practice typing is complicated by the merging of agglutinative types and the antigenic instability of meningococcus cultures.[36] Typing is essential for accurate epidemiological studies, however, and is commonly done in this connection. For routine identification agglutination with polyvalent antiserum suffices.

Studies on the nature of the antigens of the meningococcus by Rake and Scherp[37] showed that three types of antigen are present. One is a polysaccharide common to all types of meningococci and found in the gonococcus also, which was designated "C" substance. A second fraction, likewise common to all meningococcus types and highly toxic to rabbits, is protein in nature and designated "P" substance. A third fraction was type-specific and in Types I and III is polysaccharide in nature and identical in the two types. The type-specific substance from Type II appeared to be a polysaccharide-polypeptide complex.[38]

While the routine typing of meningococci is probably not worth while, typing in connection with the preparation of therapeutic antisera is obviously of importance, and at the present time such antisera are generally polyvalent.

Pathogenicity for Man.—The resistance of man to meningococcus infection is relatively high, and the incidence of healthy carriers is invariably considerably higher than that of cases of the disease. It is probable that predisposing factors play a large part in determining whether or not infection will occur; insufficient clothing, inadequate ventilation, exposure to inclement weather and fatigue very likely contribute in large measure to increasing susceptibility.

The meningococcus is initially present in the nasopharynx and from there gains access to the central nervous system. The route by which this occurs is uncertain; it is thought by some that the bacteria follow the perineural spaces of the olfactory nerves or set up a preliminary sinusitis and reach

[35] Branham and Carlin: Proc. Soc. Exp. Biol. Med., 1942, *49*:141.

[36] *Cf.* Branham, Taft and Carlin: Pub. Health Repts., 1931, *46*:897; Branham: Jour. Immunol., 1932, *23*:49.

[37] Rake and Scherp: Jour. Exp. Med., 1933, *58*:341, 361.

[38] *Cf.* Menzel and Rake: Jour. Exp. Med., 1942, *75*:437.

the brain either via the lymphatics or by direct extension through the bone. Others believe the meningococci reach the central nervous system via the blood stream through a preliminary bacteremia. While there is no definitive evidence concerning the means by which meningococci reach the central nervous system from the nasopharynx, the evidence appears to favor the hematogenous route.

In the healthy carrier the infection remains confined to the nasopharynx and in this case is short-lived there and produces few or no symptoms. When the blood stream is invaded early in the disease hemorrhages usually occur in the skin and petechiae appear, especially on the wrists and ankles, or on any mucous or serous surfaces. These are apparent in twenty-four hours after invasion and fade in a few days. The rash is quite different from other purpuric rashes; the spots take geometric shapes and are highly irregular in size. Meningococci may be observed in smears of material taken from

Fig. 58.—Meningococci in spinal fluid, showing phagocytosis of the microorganisms. Gram stain; × 1050.

these lesions.[39] Other symptoms include sudden onset, chills, fever, and meningeal symptoms such as headache, drowsiness, etc. Pain in the arms and legs is common.[40]

Upon reaching the central nervous system, the meningococcus sets up a suppurative lesion of the meninges which involves the surface of the spinal cord together with the base and cortex of the brain. The microorganisms are invariably present in the spinal fluid, which may vary from a slight to a heavy turbidity. The bacteria are found, sometimes in great numbers, both free and within the leucocytes in smears of spinal fluid. The case fatality is variable but in any case high, and ranges from 35 per cent to 80 per cent.[41]

There is some divergence of opinion regarding the nature and extent of sequelae to meningococcus meningitis, but these appear to include deafness,

[39] Tompkins: Jour. Amer. Med. Assn., 1943, *123*:31.
[40] For a general discussion see Strong: Amer. Jour. Med. Sci., 1943, *206*:561.
[41] *Cf.* Branham: Bull. Office Internat. d'Hyg. Pub., 1937, *29*:2148.

which is the most common, blindness, and pain and weakness of the neck, arms and legs. In the series of cases studied by Maddock[42] 7 to 8 per cent of the patients showed sequelae persisting for nine to thirty months after recovery.

Epidemiology.—Like most of the respiratory infections, meningococcus meningitis is disseminated by direct contact and droplet infection through the secretions of the mouth, nose and throat. Infection is spread by patients and convalescents to a limited extent, but healthy carriers of meningococci are of primary importance. Some persons are temporary carriers, while others are chronic, discharging meningococci more or less continuously or in a sporadic fashion. Of a group of ten carriers studied at the Rockefeller Institute,[43] half were of the chronic type, carrying what was apparently the

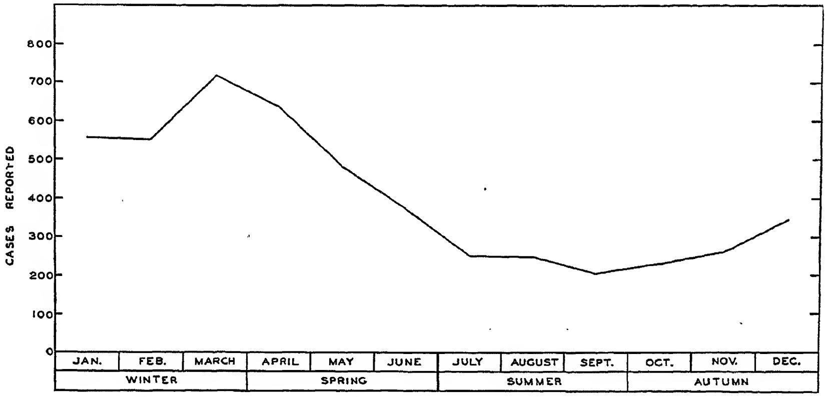


Fig. 59.—The seasonal incidence of meningococcus meningitis. Averages of reported cases by months for the years 1930 to 1938 inclusive. Data from Supplements to Public Health Reports.

same strain of microorganism for over two years. Weekly examination might be negative for a period, in one case as long as four and one-half months, and then the same type of meningococcus would appear. It is suggestive that the chronic carrier state seems often to be associated with some abnormal condition of the upper respiratory tract.

The carrier rate is, of course, variable but is undoubtedly directly related to the incidence of clinical disease. Studies[44] during the first World War indicated that the normal carrier rate in some of the troops was 2 to 4 per cent but rose preceding an epidemic, and when it reached 20 to 30 per cent cases began to appear. As the epidemic developed the carrier rate continued to

[42] Maddock: Med. Res. Council (Great Britain) Monthly Bull. Ministry of Health and Emergency Pub. Health Lab. Service, 1943, *2*:111.

[43] Rake: Jour. Exp. Med., 1934, *59*:553.

[44] *Cf.* Med. Res. Council Spec. Rept. Ser. No. 50, 1920.

[45] As in the Detroit epidemic of 1928–29. *Cf.* Norton and Gordon: Jour. Prev. Med., 1930, *4*:207.

rise, sometimes to as high as 80 to 90 per cent. The increase in carrier rate appeared in some instances to be associated with the spacing of the beds in barracks, occurring when the beds were within a few inches of one another; spacing of the beds 3 feet or more apart resulted in a reduction of the carrier rate from 30 per cent to less than 2 per cent. The general significance of these observations is uncertain, however, for in some outbreaks congestion of the population has appeared to be a minor factor,[45] in other instances the carrier rate may be 20 per cent or more without the occurrence of cases,[43] and sporadic cases occur with a low (5 per cent) carrier rate.

Epidemics of meningococcus meningitis are prone to occur in military populations, and epidemic cerebrospinal fever was, with influenza, one of the most important diseases among the troops in the first World War. The influence of predisposing factors causing an increased susceptibility is especially marked under conditions of military life; fatigue, exposure to inclement weather and similar factors very likely play a part in reducing

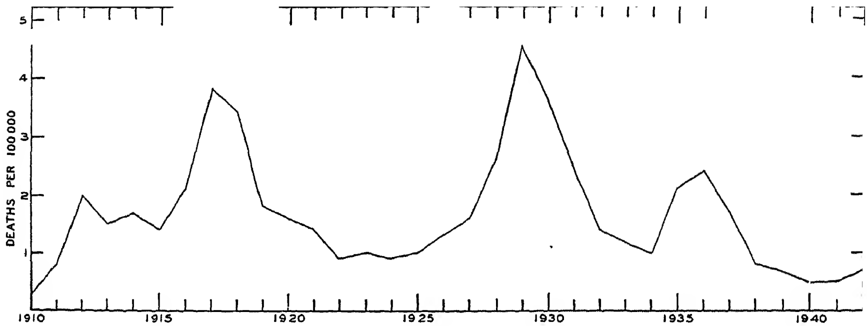


Fig. 60.—The prevalence of meningococcus meningitis in the Registration Area since 1900 as indicated by the uncorrected death rates. From data compiled by the Bureau of the Census.

the normal resistance of the raw recruit. This general reduced resistance, coupled with the opportunities for the dissemination of the microorganisms provided by the close communal existence in barracks, undoubtedly is an important factor in the genesis of these epidemics. Outbreaks in the civilian population differ in that children (over three months) and adolescents are generally attacked; susceptibility appears to be greatest in children under ten years of age, distinctly less in adolescents, and remains at a low level thereafter.

Whether occurring in civilian or military populations, epidemics of meningococcus meningitis have a number of distinctive characteristics. The relatively high carrier rate that may prevail, coupled with the low morbidity, estimated at 0.01 to 0.3 per cent of the exposed population, is an expression of a high degree of normal resistance, while the high case-fatality rate indicates the serious course of the infection once established. The relative insusceptibility of the general population also results in the spotty character of the spread of the disease; some groups escape which are closely associ-

ated with the focus of infection, while in others, apparently remote, outbreaks occur. Direct transmission from case to case is infrequently observed. Usually carriers constitute the link between cases. Further, epidemics frequently consist of a series of recurring outbreaks rather than the well-marked single epidemic wave often observed to occur in other diseases. The seasonal incidence is marked in temperate climates (see Fig. 59). Males are more frequently attacked than females, although this may be an expression of risk rather than sex differences in resistance. Racial differences in susceptibility are indicated by military experience in which the morbidity and mortality rates of the colored troops were twice that of the white.

Bacteriological Diagnosis of Meningococcus Infection.—The meningococcus is present in considerable numbers in the spinal fluid in meningitis, and the finding of the characteristic gram-negative intracellular diplococci in stained smears of the sediment from centrifuged spinal fluid is sufficient to establish a provisional diagnosis. It may be cultured by the direct inoculation of spinal fluid sediment or nasopharyngeal swabs (in the detection of carriers) on infusion base chocolate agar or blood agar. Blood culture is useful early in the disease and in those cases which do not show meningeal symptoms. In any case it is important that the specimen not be allowed to cool below body temperature before inoculation of the warm medium. Like the gonococcus, the meningococcus is oxidase-positive and the oxidase test is especially useful for nasopharyngeal cultures. The isolated meningococci may be identified by fermentation tests and agglutination with polyvalent antiserum. Typing by agglutination with type-specific antiserum may be carried out if desired.

Pathogenicity for Lower Animals.—The usual experimental animals are relatively resistant to the meningococcus upon intraperitoneal or intravenous inoculation. White mice are more susceptible than most other animals, and the injection of sufficient quantities of meningococci will result in death. Rabbits react in a similar fashion. Enormous numbers of bacteria must be injected, however, and there is some question as to whether an actual infection is set up; killed meningococci are as effective as the living cells, and it is likely that the observed result is essentially a toxemia.

Flexner[46] was able to infect *rhesus* monkeys by the intraspinal inoculation of large amounts of meningococcus cultures, and the disease appeared to be more acute than in man. Branham and her colleagues have been able to reproduce meningococcus meningitis in rabbits[47] and in guinea pigs[48] by the intracisternal injection of virulent meningococci. As in man, the experimental disease is both a local meningeal involvement with a purulent meningitis and a general toxemia. According to Branham it is easier to produce meningitis in guinea pigs than in rabbits. Similarly, Andrewes and Lush[49] have produced fatal meningo-encephalitis in mice by intracerebral inoculation though not by other routes. It is of interest to note that the

[46] Flexner: Jour. Exp. Med., 1907, *9*:142.
[47] Branham and Lillie: Pub. Health Repts., *47*:2137.
[48] Branham, Lillie and Pabst: Pub. Health Repts., 1937, *52*:1135.
[49] Andrewes and Lush: Jour. Path. Bact., 1941, *52*:85.

developing chick embryo may be infected with meningococci; the twelve-day embryos develop a septicemia and hemorrhagic lesions simulating fulminating meningococcus septicemia[50] in man.

Immunity.—Infection with the meningococcus leads to the development of demonstrable antibodies and patient's serum may agglutinate to a titer of 1 : 50. The development of agglutinins is irregular, or possibly appears so because of the antigenic instability of meningococci, and cannot be depended upon for diagnosis. Complement-fixing antibodies are produced also but too late in the disease (eight to fourteen days) to be of diagnostic utility.[51]

The injection of large numbers of meningococci into horses induces the formation of agglutinins, opsonins and amboceptors, and the horse serum has a curative value.[52] The therapeutic use of antiserum was placed upon a sound basis by the work of Flexner[53] and his collaborators. A curative serum was prepared by Flexner and Jobling[54] by injecting a horse first with gradually increasing doses of dead meningococci, then of living cocci, and finally of an autolysate. Injection into the spinal canal of this serum exerts, in many instances, a marked influence upon the course of the disease. The effect is due in part to an antitoxic action, in part to a stimulus to increased phagocytosis, and in part to a direct injurious effect upon the meningococci, for observations of the cerebrospinal fluid after injection show a remarkable destruction of the meningococci. The antiserum is apparently not particularly effective when given intravenously, but a combination of intravenous and intrathecal administration appears to be better than intrathecal injection alone. Flexner has reported[55] that in 1294 cases treated with serum the mortality was not quite 31 per cent, as compared with an average case fatality of 70 per cent in the pandemic which began in 1904 and had not wholly ended in 1913. In 199 cases in which antiserum was administered between the first and the third day of the disease the mortality was 18 per cent. Variation in the case-fatality rates in various epidemics is wide, however, and it is difficult if not impossible to determine the relative effect of differences in the virulence of the meningococci, variation in the efficacy of the sera employed and differences due to early or late administration of the serum.

The use of polyvalent antiserum is, of course, a necessity, as pointed out earlier. Continued experience with antimeningococcus serum tends to emphasize some of the difficulties of practice and interpretation. No satisfactory method for the standardization of the serum has been as yet devised; agglutinin titer has been used, and recently a standardization in terms of mouse protection has been suggested.[56] The last appears to be the best method of assay but must be carefully controlled.[57] In spite of the beneficial

[50] Buddingh and Polk: Jour. Exp. Med., 1939, *70*:485, 499.
[51] *Cf.* Cruickshank: Jour. Path. Bact., 1941, *52*:142.
[52] Kolle and Wassermann: Deut. med. Wchnschr., 1906, *32*:609.
[53] Flexner: Jour. Exp. Med., 1907, *9*:168.
[54] Flexner and Jobling: Jour. Exp. Med., 1908, *10*:141.
[55] Flexner: Jour. Exp. Med., 1913, *17*:553.
[56] Branham, Pittman, Rake and Scherp: Proc. Soc. Exp. Biol. Med., 1938, *39*:348.
[57] Pittman: Pub. Health Repts., 1941, *56*:92.

results frequently reported from the use of antiserum, there are some cases and some epidemics in which the serum treatment seems of little avail.[58]

Prophylactic vaccination with suspensions of meningococci has been attempted by a number of workers, but the procedure is difficult to evaluate. For example, Maclean and Bevan[59] found no evidence of the efficacy of active immunization in their study of an epidemic in Cyprus, while Genevray[60] has reported highly encouraging results in Tonkin. In general there appears to be no consistent and unequivocal evidence of a significant degree of protection conferred by active immunization procedures.

OTHER GRAM-NEGATIVE DIPLOCOCCI

In addition to the gonococcus and the meningococcus, two other species of non-pigmented gram-negative diplococci are generally recognized.

Fig. 61.—*Neisseria catarrhalis.* Smear from a pure culture. Note the diplococci and elongated forms which have not yet divided. Fuchsin; × 1050.

Neisseria catarrhalis is found commonly in the nasopharynx of healthy individuals as well as of persons suffering from colds and other respiratory infections. The cells as a rule are somewhat smaller than those of the meningococcus. Growth occurs on ordinary nutrient agar much more readily than in the meningococcus, and the colonies are generally thicker and more opaque. Dextrose and other sugars are not fermented. Different strains vary in their pathogenicity for animals, but many strains are fully as pathogenic as meningococci for white mice. In man they appear at times to excite catarrhal inflammation and sometimes pneumonia. They seem to have been conspicuous invaders in the 1918 influenza epidemic in some localities.

Neisseria sicca is a small gram-negative coccus found on the mucous

[58] The treatment of meningococcus meningitis is discussed by Branham: Pub. Health Repts., 1938, *53:*645.

[59] Maclean and Bevan: Proc. Roy. Soc. Med., 1939, *32:*1551.

[60] Genevray: Rev. méd. franc. d'extreme-Orient, 1941, *19:*143

membranes of the respiratory tract. It grows at room temperature as well as at 37° C., forms white, firm, dry, adherent colonies, and ferments sucrose, lactose and maltose. Although not ordinarily regarded as pathogenic, it has been found as apparently the causal agent in a case of kidney infection[61] and has been found in the blood stream of patients ill with clinical endocarditis.[62]

The Pigmented Forms.—Gram-negative diplococci which form a pale greenish-yellow pigment often best observed by transmitted light may be found in the upper respiratory tract of man. Formerly regarded as non-pathogenic, one species, *N. flavescens*, has been described as occurring in the spinal fluid in cases of clinical meningitis, as indicated earlier. These pigmented forms are differentiated from one another on the basis of fermentations.

FERMENTATION REACTIONS OF THE GRAM-NEGATIVE DIPLOCOCCI

Pigmented Species	Dextrose	Maltose	Sucrose	Levulose	Mannitol
N. perflava (*flava I*)	+	+	+	+	+
N. flava (*flava II*)	+	+	−	+	−
N. subflava (*flava III*)	+	+	−	−	−
N. flavescens	−	−	−	−	−

The Obligate Anaerobic Species.—Three species of *Neisseria* which are obligate anaerobes are recognized by Bergey,[29] *viz.*, *N. discoides*, *N. reniformis* and *N. orbiculata*. These bacteria are found as parasites on man in the mouth and in the intestinal and genito-urinary tracts but are not of known pathogenicity. It may be noted here that Bergey now includes under the family *Neisseriaceae* a new genus, *Veillonella*, made up of two species, *V. parvula* and *V. gazogenes*. These bacteria have been known as *Micrococcus parvulus* and *Micrococcus gazogenes*, and whether the creation of a new genus is justified remains to be seen.

[61] Kretschmer and Hufnagel: Jour. Amer. Med. Assn., 1924, *82*:1850.
[62] Schultz: Jour. Amer. Med. Assn., 1918, *71*:1739; Shaw: Science, 1932, *75*:488.

CHAPTER 18

THE ENTERIC BACILLI: THE COLIFORM BACTERIA AND FRIEDLÄNDER'S BACILLUS

The gram-negative, non-spore-forming bacilli make up a large group of bacteria which includes intestinal commensals such as the colon bacilli and *Proteus*; the enteric pathogens such as the typhoid, paratyphoid and dysentery bacilli; certain saprophytic forms and plant pathogens; and, as more distant relatives, the hemophilic bacteria (*Hemophilus*); the so-called hemorrhagic septicemia group (*Pasteurella*); and the causative microorganisms of undulant fever (*Brucella*).

By far the largest of these subdivisions is that of the enteric bacilli whose habitat is the intestinal tract of man. Some are, for the most part, intestinal commensals with but feeble pathogenic powers while others are highly pathogenic, producing intestinal disease of greater or lesser severity. The cholera vibrio is sometimes considered as somewhat set apart from the enteric group proper because of its curvature; this would appear to be an over-emphasis of a minor morphological difference for not only do the longer enteric bacilli often show a slight curvature, but after continued cultivation on laboratory media the cholera vibrios lose their curvature and become morphologically indistinguishable from the other enteric bacilli. Still other bacteria are closely related to the enteric bacilli, in fact sometimes cannot be distinguished from them with certainty, but are set apart by differences in habitat. Such are Friedländer's bacillus, a common inhabitant of the upper respiratory tract and the causative agent of a small proportion of pneumonias, and the plant pathogens of the genus *Erwinia* which produce soft rots of vegetables. Similarly, the members of the genera *Proteus* and *Pseudomonas* occur as free-living saprophytes as well as intestinal commensals of occasional pathological significance.

The enteric bacilli fall into certain natural groups on the basis of physiological, immunological and pathogenic characteristics. These are: the coliform group which includes *Bacterium coli* and *Bacterium aerogenes* together with intermediate and variant forms; the *Salmonella* group made up of the typhoid and paratyphoid bacilli; the group of dysentery bacilli; and the cholera and paracholera vibrios. The differences between these groups, however, are differences of respective modal conditions of the differential characters, and at their peripheries the groups merge into one another to give a continuous series of intergrading types whose taxonomic positions and relationships have so far defied precise and satisfactory definition.[1] A useful

[1] Because of the lack of better general agreement than now exists regarding the taxonomy of these forms, the writer prefers to retain the generic name *Bacterium* for many of the enteric bacilli.

primary differentiation can be made on the basis of the lactose fermentation which is roughly correlated with pathogenicity; the coliform bacteria ferment this sugar rapidly with the formation of acid and gas in twenty-four hours, while the bacteria of the other groups, essentially pathogens, do not ferment it. The distinction is not absolute, of course, since both the paracolon bacilli and certain of the dysentery bacilli are slow lactose fermenters, but is sufficiently marked to have considerable practical value.

The lactose-fermenting bacteria constitute the subject matter of the present chapter, and the non-lactose-fermenters will be discussed in the chapters immediately following.

BACTERIUM COLI

Bacterium coli (*Escherichia coli*) was described by Escherich[2] in 1886 under the name of *Bacterium coli commune*. The original culture was isolated from the dejecta of a breast-fed infant, and cultures from this source were considered by Escherich to be especially typical. *Bact. coli* is, however, widely distributed, although "ubiquitous" only in the limited sense that it is universally found in the intestinal tract of man and many of the higher animals. It is especially abundant in the colon and is so characteristic an inhabitant of this region of the intestine as fully to deserve the name that has been given it. From fresh, healthy human feces it is often isolated in pure culture by the ordinary aerobic methods, although microscopic examination shows that other kinds of microorganisms are also present.

Morphology and Staining.—The colon bacillus exhibits considerable variation in its morphology. The usual dimensions observed in stained preparations from cultures upon nutrient agar or gelatin range from 2 μ to 4 μ in length and from 0.4 μ to 0.7 μ in breadth. Very short, oval and coccus-like forms are not infrequently found and usually predominate when the bacillus is observed directly in the normal animal. The bacilli are occasionally observed in pairs or short chains. Some varieties are encapsulated, particularly those found in pathological conditions. Motility is variable, although the most typical strains are motile by peritrichous flagella. Spores are not formed.

Colonies upon nutrient gelatin are more consistent and characteristic in appearance than those upon agar media. They are opaque to partially translucent, smooth, moist and homogeneous in consistency, with entire to undulating edge, and exhibit the maple-leaf appearance common to many of the enteric bacteria (see below). Colonial morphology is somewhat variable upon nutrient agar. Typical colonies are opaque and grayish-white, and there may be a tendency to become a light yellowish brown upon continued incubation. Pigmented varieties are occasionally observed.[3] In certain differential media the colonies of *Bact. coli* may assume other characteristics typical under the special circumstances. Upon Endo's medium, for example, the colonies are, of course, red but, in addition, take on a curious metallic sheen that is highly characteristic when viewed by reflected light. Some

[2] Escherich: *Die Darmbacterien des Säuglings*. Stuttgart. 1886.
[3] *Cf.* Gililland and Reese: Jour. Bact., 1943, *45*:499.

varieties are β-hemolytic on blood agar; such hemolytic strains occur with much greater frequency in pathological processes than in the normal intestine.

Bact. coli stains readily with the ordinary aniline dyes and is gram-negative. Flagella may be demonstrated by special staining methods.

COMPARATIVE DIFFERENTIAL REACTION OF IMPORTANT INTESTINAL BACTERIA*

	Dextrose	Lacrose	Sucrose	Rhamnose	Mannitol	Salicin	Maltose	Xylose	Arabinose	Sorbitol	Trehalose	Dulcitol	Raffinose	Inositol	Indol	Gelatin	Milk	Production of Hydrogen Sulfide	Russell's Medium		Tartrate.†
																			Butt	Slant	
Bacterium coli communior	G	G	G	G	G	—	G	G	G	G	G	±	G	—	+	—	ac	—	G	a	
Bacterium coli communis	G	G	—	G	G	G	G	±	G	G	G	±	±	—	+	—	ac	—	G	a	
Bacterium aerogenes	G	G	G	G	G	G	G	G	G	G	G	±	G	G	—	—	ac	—	G	a	
Bacterium friedländeri	G	±	G	G	G	G	G	G	G	G	G	G	G	—	±	—	a	—	G	a	
Bacterium cloacae	G	G	G	G	G	G	G	G	G	G	G	±	G	a	±	+	ac	—	G	ak	
Salmonella paratyphi *A*	G	—	—	G	G	—	G	—	G	G	G	G	—	—	—	—		—	G	k	k
Salmonella paratyphi *B*	G	—	—	G	G	—	G	G	G	G	G	G	—	$\overset{a}{-}$	—	—	ak	+	G	k	k
Salmonella typhi-murium	G	—	—	G	G	—	G	G	G	G	G	G	—	±	—	—	ak	+	G	ak	a
Salmonella enteritidis	G	—	—	G	G	—	G	G	G	G	G	G	—	—	—	—	ak	+	G	ak	a
Salmonella choleraesuis	G	—	—	G	G	—	G	G	—	G	—	±	—	—	—	—	ak	±	G	ak	a
Salmonella abortivo-equinus	G	—	—	G	G	—	G	G	G	G	G	G	—	—	—	—	ak	—	G	ak	a
Salmonella paratyphi *C*	G	—	—	G	G	—	G	G	G	G	G	G		—	—	—	ak	+	G	ak	a
Salmonella pullorum	G	—	—	G	G	—	—	—	G	G	—	—	—	—	—		ak	—			
Salmonella gallinarum	+	—	—	a	a	—	a	a	a		a	a	—		—	—	ak	—			
Bacterium typhosum	a	—	—	—	a	—	a	$\overset{a}{-}$	—	a	a	—	—	—	—	—	ak	+	a	k	
Bacterium flexneri	a	—	$\overset{a}{-}$	a	a	$\overset{a}{-}$	$\overset{a}{-}$	$\overset{a}{-}$	$\overset{a}{-}$	$\overset{a}{-}$	a	$\overset{a}{-}$	$\overset{a}{-}$	$\overset{a}{-}$	±	—	ak	—	a	k	
Bacterium shigae	a	—	—	—	—	—	—	—	—	—	$\overset{a}{-}$	—	—	—	—	—	ak	—	a	k	
Bacterium sonnei	a	a	a	a	a	a	a	—	a	—	a	—	a		—	—	ac	—			
Alcaligenes fecalis	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	k	+	—	k	

* G, acid and gas; a, acid only; ac, acid and coagulation; $\overset{a}{-}$, variable (acid or negative); +, positive (indol production, gelatin liquefaction or H_2S formation); —, no change; ±, variable (acid and gas or negative); k, alkaline; ak, acid to alkaline.

† The tartrate reactions (Jordan and Harmon: Jour. Inf. Dis., 1928, *42*: 238) have been found useful in differentiating within the Salmonella group but have not been tested on the other types.

Physiology.—The colon bacillus is a facultative anaerobe, growing equally well under aerobic or completely anaerobic conditions. It grows luxuriantly upon the ordinary nutrient media and may be cultivated in synthetic solutions containing an ammonium salt and an organic source of carbon such as glucose. Growth occurs over a temperature range of 10° to 46° C., there is good growth from 20° to 40° C., and the optimum is 37° C.

Milk is curdled with an acid reaction, usually within forty-eight hours. Gelatin is not liquefied, but indol is produced in abundance by cultures in peptone water. Hydrogen sulfide is produced in only very small amounts; cultures in the usual iron or lead acetate media are negative, but more sensitive methods give a positive reaction.

Various sugars are actively fermented with the production of acid and gas, including dextrose, lactose, maltose, arabinose, xylose, rhamnose and mannitol. The fermentation of other sugars, including sucrose, is variable, but the polysaccharides dextrin, starch and glycogen are not fermented. The gas produced is a mixture of carbon dioxide and hydrogen, generally in a ratio of 1 : 1. The greater part of the acid produced is lactic acid; smaller quantities of formic and acetic acids are formed, together with ethyl alcohol. Succinic acid is also found in variable but small amounts.

The colon bacillus is of ordinary resistance to deleterious influences, being, as a rule, neither as resistant as staphylococci nor as susceptible as the more delicate bacteria. Most strains are killed by exposure to 60° C. for thirty minutes, but occasionally more resistant varieties are encountered. In common with the other gram-negative bacteria, it is considerably less susceptible to the bacteriostatic action of dyes than are the gram-positive microorganisms, and selective media containing dyes are commonly used in the primary isolation of the enteric bacteria. The ability of the colon bacillus to grow in the presence of bile is likewise made use of in differential media (MacConkey's broth) in the bacteriological examination of water.

Variation.—The existence of two colony types of *Bact. coli* has long been known, the one the flat, maple-leaf form, and the other a smaller, raised, round, moist type. The colon bacillus dissociates into rough and smooth colonial types,[4] the S form giving rise to smooth, round, domed, shiny, translucent colonies, while the R colony type is characterized by an irregular, dull surface, jagged outline and opacity. It has been suggested[4] that the R form is the more virulent.

Toxins.—As in the case of many other bacteria, the cell substance is toxic to experimental animals upon parenteral inoculation. The specificity of the endotoxin has not been established. Vincent[5] has described two toxic substances formed by the colon bacillus, the one a heat-labile neurotropic toxin and the other a heat-stable enterotropic toxin. An enterotoxic substance that has been reported by other workers[6] is of interest in connection with the epidemiological implication of colon bacilli in outbreaks of food poisoning. Some strains of *coli* produce filterable hemolysin.

Pathogenicity for Man.—The ability of the colon bacillus to set up pathologic processes in man is very slight. The microorganism is constantly present in the intestinal tract and, although excessive sugar fermentation with the liberation of irritant acids and gas may possibly be responsible for some cases of diarrhea, in general it does little harm. The term pathogenicity is, as has been pointed out elsewhere, a relative one, and *Bact. coli*

[4] Gratia: Jour. Exp. Med., 1922, *35*:287.

[5] Vincent: Compt. Rend. Acad. Sci., 1925, *180*:239, 407, 1083, 1624; see also Weinberg and Prevot: Compt. Rend. Soc. Biol., 1927, *97*:164.

[6] Jordan and Burrows: Jour. Inf. Dis., 1935, *57*:121.

may, on occasion, invade the body tissues and set up a focus of infection. The urinary tract is probably the most frequently invaded, and the majority of cases of cystitis are of *coli* etiology. The colon bacilli may also play a part in the formation of gallstones; the bacillus is frequently found in the core of gallstones and, in culture, can precipitate cholesterol and other biliary constituents. The injection of colon bacilli into the healthy urinary bladder or gallbladder does not produce infection in experimental animals, but infection occurs if the bile duct or urethra is obstructed. Local infections such as abscesses, conjunctivitis and the like have been observed but are not common. *Bact. coli* septicemia is very rare but may occur as an agonal invasion in acute infective processes. A hemorrhagic septicemia caused by *Bact. coli* sometimes occurs in newborn children and is known as Winckel's disease.

In general, however, *coli* infection is rare except in cystitis, and it is probable that the pathogenic powers of the colon bacillus have been exaggerated, particularly by the early writers who in many instances failed to differentiate *coli* from other members of the enteric group. It may be noted that the common occurrence of agonal or postmortem invasion of the body by the colon bacillus tends to diminish the value of evidence derived from finding this bacterium in the internal organs after death.

Pathogenicity for Lower Animals.—*Bact. coli* is of low pathogenicity for laboratory animals. Two cubic centimeters of a broth culture injected intraperitoneally will kill a guinea pig within a few days, and killed bacilli are very nearly as effective. Spontaneous infection of lower animals is not common. The diarrhea of young calves known as "scours" has been attributed to *Bact. coli* septicemia. The colon bacillus is also thought to be a factor in the causation of diarrhea of foals and young pigeons. Local infection may occur; in one instance of 286 cases of bovine mastitis, 10 were apparently due to members of the colon-aerogenes group, of which 3 were typical *Bact. coli.*[7]

Varieties of Bacterium Coli.—The above discussion has been one of "typical" *Bact. coli* and, although the description holds true in general, "atypical" strains are not infrequently found. These varieties may be separated, for pedagogical purposes, into two groups, one "fermentatively atypical," and the other, the strains intermediate between *Bact. coli* and *Bact. aerogenes*. The latter straddle the lines of demarcation drawn between the two species by various differential tests and will be discussed in a later section in that connection.

The Fermentative Varieties.—These may, of course, be many, owing to the number of sugars whose fermentation by these bacteria is variable. A few of these are, however, well known and may be mentioned briefly. In regard to the sucrose fermentation, about half of the strains of *coli* isolated are able to ferment this disaccharide while the other half are not, and the two fermentative types have been given different names; the strains of *Bact. coli* that ferment sucrose are called *Bacterium coli communior*, while those that do not ferment sucrose are termed *Bacterium coli communis* (or *commune*).

[7] Gwatkin, LeGard and Hadwen: Canadian Jour. Comp. Med., 1938, 2:155.

Much less frequently strains of *Bact. coli* are isolated which do not immediately ferment lactose, *i.e.*, their colonies on Endo's medium are white. In the course of incubation, however, red papillae develop on the white colonies which, upon subculture, ferment lactose and breed true. Such strains have been known for many years and are termed *Bacterium coli mutabile*. This phenomenon is discussed elsewhere (p. 146) in connection with bacterial variation.

A fourth fermentative type which resembles *Bact. coli* in all particulars except that it ferments sugars with the production of acid and no gas is generally known as *Bacterium coli anaerogenes*. Although these names are commonly used they have no taxonomic standing and are a matter of convenience only.

The Paracolon Bacilli.—Another fermentative variety whose relationship to the typical coliform bacteria is not clear is that which is characterized by late (five to fourteen days) lactose fermentation. In this respect these strains resemble the Sonne and *dispar* dysentery bacilli, and some strains resemble the typhoid and dysentery bacilli in that they are anaerogenic. They may be grouped into paracolon-coli, -intermediate, and -aerogenes strains.[8] They are also intermediate in that some strains have been found to contain somatic or flagellar antigens of the *Salmonella* group and the Vi antigen of the typhoid bacillus.[9] They appear to be pathogenic to a minor degree, not only having been found in association with mild enteric infection, but also having produced laboratory infections. They are perhaps to be regarded as transitional forms between the lactose-fermenters and the non-lactose-fermenters and possibly allied to the Sonne dysentery bacilli. The group is, however, a heterogeneous one and is not to be regarded as a well-defined type of enteric bacteria.

Bacterium cloacae, regarded by many as a distinct species, may be mentioned here, for it differs from *Bact. coli* in that indol formation is variable but gelatin is liquefied. The latter property serves to set *Bact. cloacae* off from the other members of the colon-aerogenes group, and it may be properly regarded as an intermediate form connecting the colon bacilli with the *Proteus* group, which do not ferment lactose but actively liquefy gelatin. *Bact. cloacae* has also been found to have some pathogenic properties.[10]

BACTERIUM AEROGENES

Escherich originally described two types of gram-negative lactose-fermenting bacilli, the one *Bact. coli* and a second which was nonmotile, somewhat shorter and plumper, and which clotted milk more rapidly. This second variety, first termed *Bacterium lactis aerogenes*, is now generally known as *Bacterium aerogenes* (*Aerobacter aerogenes*). *Bact. aerogenes* is commonly found in soured milk and, unlike *Bact. coli*, appears to live a saprophytic existence in nature on the surface of grains and similar places. Because of the apparent difference in the origin of these two kinds of lactose-

[8] See Stuart, Wheeler, Rustigan and Zimmerman: Jour. Bact., 1943, *45:*101; Stuart and Rustigan: Amer. Jour. Pub. Health, 1943, *33:*1323.

[9] See the discussion of Peluffo, Edwards and Bruner: Jour. Inf. Dis., 1942, *70:*185

[10] Caminita *et al.:* Pub. Health Repts., 1943, *58:*1165.

fermenters, their differentiation has been a matter of great interest in connection with the use of *Bact. coli* as an indicator of fecal pollution (p. 223).

Because of the close resemblance of *Bact. coli* and *Bact. aerogenes*, the latter may be adequately described in terms of its differences from the former. The morphological differences are minor and variable and cannot be used as differential characters. *Bact. aerogenes*, it may be noted, is often encapsulated, while the presence of capsules on *Bact. coli* is relatively infrequent. In general, the fermentative power of *Bact. aerogenes* is somewhat greater than that of *Bact. coli;* the ratio of carbon dioxide to hydrogen in the gas produced is about 2 : 1, milk is curdled more rapidly, and starch is fermented. Growth in gelatin is more luxuriant; in gelatin tubes a projecting "nail-head" growth is characteristically produced. Indol is not produced in peptone solution.

In conjunction with the above differences, three additional tests are commonly used in the differentiation of these bacteria:

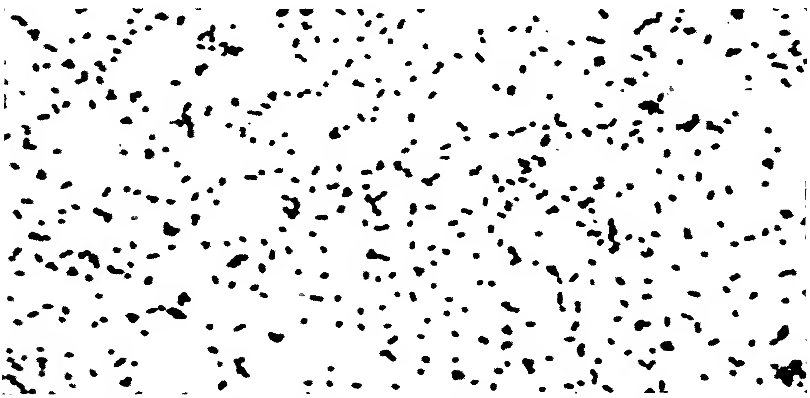


Fig. 62.—*Bacterium aerogenes.* Smear from a pure culture. Note the coccobacillary form. Fuchsin; × 1050.

(1) *The methyl red test* is no more than the determination of the *p*H of a dextrose broth culture after four to five days' incubation. *Bact. coli* produces and maintains a high acidity, and when the indicator is added it is red and *Bact. coli is said to be methyl red positive.* In *Bact. aerogenes* cultures, on the other hand, the hydrogen ion concentration is lower, the added indicator is yellow, and *Bact. aerogenes is said to be methyl red negative.*

(2) *The Voges-Proskauer reaction* is a qualitative test for the presence of acetylmethylcarbinol or, more properly, for diacetyl. In the fermentation of glucose *Bact. aerogenes* forms acetylmethylcarbinol along with organic acids and other products, while *Bact. coli* does not form this substance. The culture is grown in glucose-peptone broth for four to five days. When 5 cc. of 10 per cent potassium hydroxide solution are added a deep pink color develops on standing in cultures of *Bact. aerogenes* but not in cultures of *Bact. coli. Bact. aerogenes is,* then, Voges-Proskauer positive and *Bact. coli*

is negative and there is a negative correlation between the Voges-Proskauer test and the methyl red test. The mechanism of the Voges-Proskauer test is as follows: upon standing in the presence of alkali the acetylmethylcarbinol present is oxidized to diacetyl, which in turn combines with an unknown constituent of the peptone to form the colored substance. Acetylmethylcarbinol is not formed during the early stages of fermentation by *Bact. aerogenes* but as a consequence of further decomposition of the initial products of fermentation; hence a four- to five-day culture must be used.

(3) The *citrate test* is dependent upon the ability of *Bact. aerogenes* to utilize sodium citrate as a sole source of carbon in a synthetic medium. A medium consisting of sodium ammonium phosphate, potassium phosphate, magnesium sulfate and sodium citrate is inoculated with the bacteria. *Bact. aerogenes* grows in this solution and is said to be citrate-positive; *Bact. coli* does not grow to any appreciable extent and is said to be citrate-negative.

The differential reactions of *Bact. coli* and *Bact. aerogenes* may be summarized in tabular form:

	$CO_2:H_2$	Indol	M. R.	V. P.	Citrate
Bact. coli	1:1	+	+	−	−
Bact. aerogenes	2:1	−	−	+	+

The four most commonly used tests, indol, methyl red, Voges-Proskauer and citrate, are frequently referred to by the mnemonic "Imvic" or "IMViC," which fixes them in order.[11] In this terminology typical *coli* is (+ + − −) and typical *aerogenes* (− − + +).

Other criteria have been used from time to time, including the fermentation of cellobiose, inosite, glycerol and other carbohydrates, the utilization of uric acid and the Eijkman test. The last consists in the fermentation of glucose at 46° C. *Bact. coli* is positive to this test, while *Bact. aerogenes* is negative.

The Colon-Aerogenes Intermediates.—"Typical" *Bact. coli* and "typical" *Bact. aerogenes* are, as indicated above, readily differentiated, but these bacteria represent extremes which are connected by a variety of intergrading forms. On the basis of the IMViC tests 16 combinations are possible, and many of these have been found; Parr,[12] for example, has found 14 of these possible combinations. The allocation of these intermediate forms to *coli* or *aerogenes* is, obviously, a difficult matter and probably not advisable. It is the practice of many to set up a "*coli* group," an "*aerogenes* group" and an "intermediate group" which may be referred to in the aggregate as the *coliform bacteria.* The existence of the intergrading types so lumped and the atypical strains of *coli* discussed earlier has interesting phylogenetic implications that cannot be considered here.

[11] *Cf.* Parr: Amer. Jour. Pub. Health, 1936, *26*:39.

[12] The relationships of the coliform bacteria are discussed at length in the review by Parr: Bact. Rev., 1939, *3*:1. See also Vaughn and Levine: Jour. Bact., 1942, *44*:487.

The Immunological Relationships of the Coliform Bacteria.—The antigenic structure of the colon bacilli has been investigated by a number of workers and these bacteria have been found to be immunologically heterogeneous; it has been reported, however, that hemolytic strains of *Bact. coli* isolated from urinary infections are immunologically related.[13] Wallick and Stuart[14] have made the interesting observation that while most of the colon bacilli harbored by a given individual were immunologically identical, or nearly so, individuals showed a continuous succession of types, each predominating for a few weeks or months and then being replaced by a fresh type. It was noted also that immunologically identical types were sometimes biochemically different. Although the coliform bacteria are, on the whole, highly variable in antigenic structure and the immunological method is of no help in their differentiation, certain interrelationships may be observed.[15] Some of the coliform bacteria are immunologically related to some of the other gram-negative bacilli, such as the plant pathogens,[16] Friedländer's bacillus[17] and *Salmonella*,[18] while others appear to be related to certain of the pneumococcus types.[17, 19] The immunological relationships of the paracolon bacilli have been discussed earlier.

PERCENTAGE DISTRIBUTION OF COLIFORM TYPES*

Source	Coli	Aer.	Int.	Irr.	Total
Milk	28.8	49.5	20.3	1.4	2224
Water	51.6	28.6	18.5	1.3	9496
Soil	23.8	54.3	18.8	3.1	1330
Grains	17.9	73.8	7.3	1.0	587
Feces	87.9	5.2	6.8	0.1	3974

* As compiled from various authors. In many instances the strains studied were not isolated at random; the percentages are, therefore, weighted, and great significance cannot be attached to them.

The Ecology of the Coliform Bacteria.—The distribution of the coliform bacteria in nature is a matter of some importance in connection with the use of these organisms as indicators of fecal pollution and has been studied by a number of workers. The results of these studies are most conveniently expressed in the above tabular form modified from Griffin and Stuart.[20]

Although the percentages given there may not be regarded as an accurate expression of the distribution of the coliform bacteria in nature, it will be

[13] Dudgeon, Wordley and Bawtree: Jour. Hyg., 1922, *21*:168.
[14] Wallick and Stuart: Jour. Bact., 1943, *45*:121.
[15] *Cf.* Stuart, Baker, Zimmermann, Brown and Stone: Jour. Bact., 1940, *40*:101.
[16] Stanley: W. Virginia Agr. Exp. Station Bull. No. 287, 1938.
[17] Julianelle: Jour. Immunol., 1937, *32*:21.
[18] Gard and Eriksson: Ztschr. f. Hyg. u. Infektionskr., 1939, *122*:54.
[19] Barnes and Wight: Jour. Exp. Med., 1935, *62*:281.
[20] Griffin and Stuart: Jour. Bact., 1940, *40*:83.

clear that no strict lines of demarcation regarding habitat may be drawn. *Aerogenes* and intermediate types are found in the intestinal tract in appreciable proportions, but it is probable that *coli* found in water, soil and elsewhere in nature is present as contamination and is *always* fecal in origin.

FRIEDLÄNDER'S BACILLUS

A heavily capsulated bacterium, closely related to the coliform bacilli,[21] was described by Friedländer[22] in 1883 as the causative agent of pneumonia. As indicated in a previous chapter, the pneumococcus is the causative bacterium in the great majority of lobar pneumonias, but the bacillus described by Friedländer is also responsible for a small proportion of pneumonias in man. The bacterium has been variously known as the *pneumobacillus*, *Bacterium pneumoniae*, *Bacterium friedländeri*, *Bacillus mucosus capsulatus*, *Encapsulatus pneumoniae* and *Klebsiella pneumoniae*.

Morphology and Staining.—Friedländer's bacillus is a short, thick oval rod 1 to 2 μ long and 0.5 to 0.8 μ thick, arranged singly and in pairs end

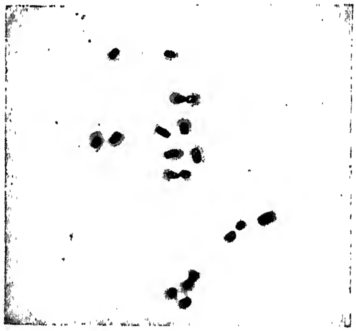


Fig. 63.—Friedländer's pneumobacillus showing the capsules; blood-agar culture; fixed with methyl alcohol and stained with carbol gentian violet (Wherry).

to end. The diplobacillus arrangement is common in the body. Some strains are pleomorphic in culture, showing filaments and other forms, but these are not usual. These bacteria are non-motile and non-spore-forming. A heavy capsule is present on the bacilli in the body and is particularly evident in cultures on the richer media such as blood agar or media containing sugar.

A slimy growth is produced on artificial media, and the colonies are round, amorphous, raised with a glistening surface and mucoid in consistency. There is no hemolysis on blood agar.

The Friedländer bacilli stain readily with the usual aniline dyes, and the bacteria are gram-negative. The capsule may be demonstrated in stained smears by special methods.

Physiology.—These bacteria are facultative anaerobes whose optimum temperature is 37° C. and temperature growth range from 12° to 43° C.

[21] See the comparative study by Osterman and Rettger: Jour. Bact., 1941, *42*:699.
[22] Friedländer: Fortschr. d. Med., 1883, *1*:715.

Nutritive requirements are exceedingly simple; these microorganisms grow luxuriantly upon the ordinary nutrient media and in simple synthetic solutions. In the writer's laboratory representatives of all the types of Friedländer's bacillus have been found to grow well in the presence of an ammonium salt and simple carbon compounds such as acetate and, in one instance, formate.

Fermentation reactions are highly variable from strain to strain, but in general a number of sugars are fermented. The lactose fermentation is variable. Gelatin is not liquefied and indol production is variable. In view of the relation of these bacteria to the coliform group it may be noted that the methyl red and citrate tests are positive, while the Voges-Proskauer test is negative.

The Friedländer bacilli are killed by exposure to 55° C. for thirty minutes but are unusually resistant to drying and are said to survive for a period of months. They are of average resistance to the usual germicidal chemicals.

Variation.—These bacteria dissociate as rough and smooth forms. The commonly observed colony type is regarded as the smooth form although analogous to the "mucoid" phase observed in some other bacteria. As in the case of the pneumococcus, the S-R transformation is associated with the loss of the capsule and with a loss of immunological type-specificity. The smooth form is virulent and the rough form avirulent.

Classification.—Friedländer's bacillus is closely related to the coliform bacteria, although how closely is uncertain. In the current Bergey classification these microorganisms are allocated to the genus *Klebsiella*, the other two in the family *Eschericheae* being *Escherichia* and *Aerobacter*, *i. e.*, the coliform bacilli. It is perhaps better for the present not to insist on such a close relationship but to regard them as members of the broad genus *Bacterium*.

Types.—In spite of a number of attempts it has not been possible to make use of fermentations or other biochemical reactions in subdividing *Bact. friedländeri*. Through the work of Julianelle,[23] however, it has been found that these bacteria may be divided into sharply defined immunological types. Analogous to the situation in the pneumococci, two types of antigen are present. The one, apparently nucleoprotein in nature, is species-specific, and the other, a polysaccharide present in the capsule, is type-specific. Julianelle has differentiated three serological types, Types A, B and C, to which the majority of strains belong, and a Group X for the remainder. Type B is immunologically similar to, although not identical with, Type II pneumococcus. The relative proportion of these types found in nature is indicated by Julianelle's[24] study of 80 strains; 42 belonged to Type A, 12 to Type B, 7 to Type C and 19 to Group X. The Type A strains were largely from human sources and the Type B strains from lower animals. He has also reported[25] that in Friedländer's bacillus pneumonias, Type A was found in 64 per cent, Type B in 14 per cent, Type C in 7 per cent, and Group X in 15 per cent.

[23] Julianelle: Jour. Exp. Med., 1926, *44:*113, 683, 735.
[24] *Ibid.*, 1930, *52:*539.
[25] Julianelle: Ann. Int. Med., 1941, *15:*190.

Pathogenicity.—Microorganisms of this group are not infrequently found associated with various upper respiratory infections in man, though it is likely that in most instances they are secondary invaders. They are commonly present in the nasopharynx of persons suffering from chronic sinusitis or chronic lung infections such as bronchiectasis. Pneumonia due to Friedländer's bacillus is rare and makes up less than 1 per cent of all pneumonias, but the case-fatality rate is high, 90 per cent or more.[26] Members of this group have also been associated with suppurative conditions in various parts of the body, such as liver abscess[27] and, rarely, have been found to invade the blood stream and give rise to septicemia.

Friedländer's bacillus infection may also occur spontaneously in lower animals and has been incriminated in a spontaneous respiratory epidemic in mice, a paralytic disease of moose and metritis of mares. Experimentally the virulence of Julianelle's types differs; mice are highly susceptible to

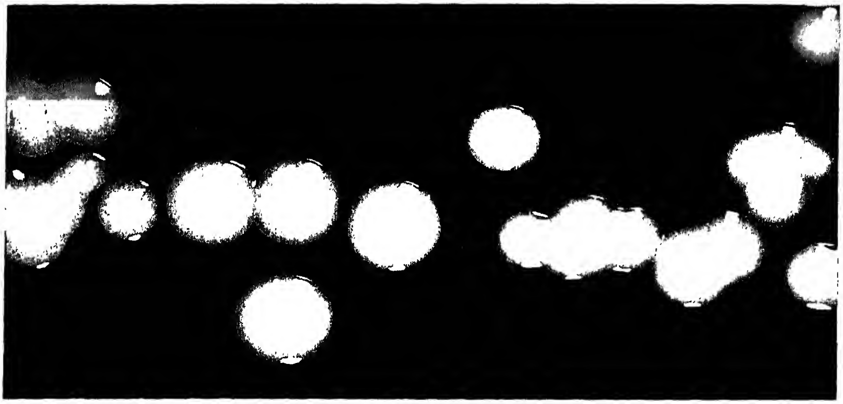


Fig. 64.—Colonies of Friedländer's bacillus on blood agar. Note the large size and mucoid appearance. × 3.

Types A and B, rabbits and guinea pigs are somewhat more resistant. Type C is relatively avirulent.

Related Bacteria.—Two other bacteria, closely related to Friedländer's bacillus, have been considered by some to be associated with specific infections. A bacillus found in a fetid catarrhal condition of the nose known as ozena closely resembles *Bact. friedländeri,* as does the so-called rhinoscleroma bacillus found in the granulomatous lesions of a rare disease of the upper respiratory tract termed rhinoscleroma. Julianelle has found that the latter microorganism is identical with Type C Friedländer's bacillus,[28] but *Bact. ozenae* is immunologically distinct from both *Bact. friedländeri* and the rhinoscleroma bacillus.[29]

[26] See the discussion by Hyde and Hyde: Amer. Jour. Med. Sci., 1943, *205:*660.
[27] *Cf.* Kinney and Ginsberg: New England Jour. Med., 1943, *228:*145.
[28] Morris and Julianelle: Jour. Inf. Dis., 1934, *55:*150.
[29] Julianelle: Jour. Bact., 1935, *30:*535.

CHAPTER 19

THE ENTERIC BACILLI: THE SALMONELLA GROUP

Family: *Enterobacteriaceae*. Tribe: *Salmonelleae*. Genus: *Salmonella*. Gram-negative, non-spore-forming rods, morphologically similar to *Bacterium coli* and serologically related to the typhoid bacillus. Dextrose is fermented, usually with gas production; lactose and sucrose not fermented. Gelatin is not liquefied and indol is not produced. Some species pathogenic for man, others for animals. Type species: *Salmonella cholerae-suis*.

The first member of this large group was described in 1888 by Gärtner,[1] who isolated it from diseased beef responsible for an outbreak of gastroenteritis, and named it *Bacillus enteritidis*. Similar bacteria have been found frequently in foods epidemiologically implicated in outbreaks of food poisoning, others are responsible for outbreaks of disease in rats, mice and other rodents, others are found in the paratyphoid fevers in man, and still others are responsible for certain poultry diseases.[2] These bacteria are parasitic and, although widely distributed in nature, do not maintain a saprophytic existence.

Morphology and Staining.—These bacilli are gram-negative rods closely resembling and indistinguishable from the coliform bacteria. They stain readily with the usual dyes such as methylene blue and carbol fuchsin. No particular arrangement of the cells is apparent on microscopic examination. All species except *S. pullorum* and *S. gallinarum* are actively motile by means of peritrichous flagella. No capsules are apparent and spores are not formed.

Physiology.—The bacteria of this group have simple nutritional requirements, growing readily on the usual nutrient media. In synthetic media an ammonium salt and glucose, pyruvate, lactate, etc. are adequate sources of nitrogen and carbon, and none of the bacterial vitamins is required. The optimum temperature is 37° C. but growth occurs at a reasonable rate at room temperature. They are facultative anaerobes, growing equally well under either aerobic or anaerobic conditions, and some species develop relatively strong reducing intensities (p. 85).

The group is characterized biochemically by failure to ferment lactose or salicin, and inability to liquefy gelatin or produce indol. There are a very few exceptions to the two last; *S. eastbourne* and some strains of *S. enteritidis* and *S. panama* produce indol, and *S. dar-es-salaam* liquefies gelatin. The evolution of gas commonly accompanies the fermentation of sugars though anaerogenic strains of *S. enteritidis*, *S. typhi-murium* and *paratyphi C* have been reported.

In the past considerable significance has been attached to the differential

[1] Gärtner: Korresp. d. allg. ärtzl. Vereins von Thüringen, 1888, *17*:233.

[2] For a comprehensive discussion of these bacteria see Kauffmann: *The Bacteriology of the Salmonella Group*. Munksgaard. Copenhagen. 1941.

biochemical reactions of these bacilli and they have been separated on this basis. In recent years, however, the antigenic structure of these bacteria has been emphasized and antigenic analysis has been carried further with this group than with any other bacteria. The differential value of sugar fermentations and other biochemical reactions remains of primary importance nevertheless. The biochemical reactions of some of the more frequently encountered *Salmonella* species are given in the accompanying table (p. 371).

Toxins.—No soluble toxin is formed by the bacteria of the *Salmonella* group, but, as is the case of many other bacteria, their cell substance is toxic upon parenteral inoculation. This toxic quality of two species, *S. enteritidis* and *S. aertrycke*, has been investigated and found to be a property of contained gluco-lipoids (p. 251 and p. 398) which have immunological individuality (somatic antigens) and hence are, in a sense, specific. Pharma-

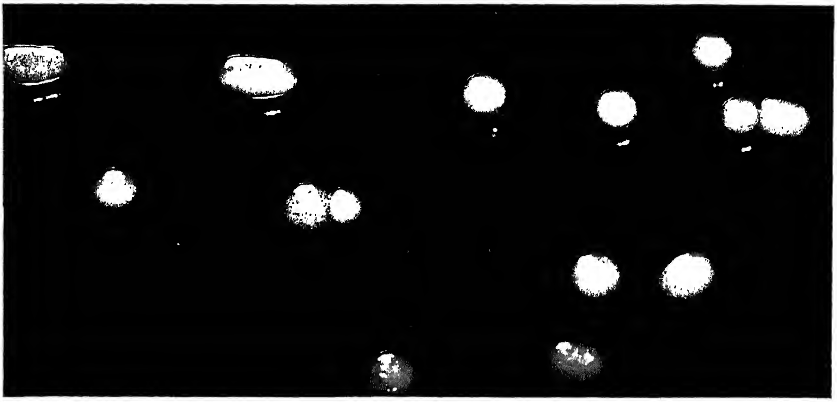


Fig. 65.—Colonies of *Salmonella typhi-murium* (*aertrycke*) on nutrient agar. Twenty-four-hour culture. × 3.

cologically, however, they are not specific, and their activity does not differ to a marked degree from that of the cell substance of other bacteria.[3]

The Immunological Differentiation of Salmonella Bacilli.—The techniques of antigenic analysis have been developed through the efforts of White[4] in the study of the *Salmonella* group, and the antigenic composition of bacteria of this group is, perhaps, better known than that of any other bacteria. Many members of the group are immunologically complex; whether because they are innately so or because they are better known in this respect than most other bacteria is difficult to say.

Flagellar and Somatic Antigens.—Two types of antigens are present in the bacteria of the *Salmonella* group, one associated with the cell substance and the other with the flagella. Early noted by Smith and Reagh,[5]

[3] See Cameron, Delafield and Wilson: Jour. Path. Bact., 1940, *51*:223.
[4] White: Med. Res. Council Spec. Rept. Series No. 103, 1926.
[5] Smith and Reagh: Jour. Med. Res., 1903, *10*:89.

the first was termed *somatic antigen* and the second *flagellar antigen*. These types were later observed by Weil and Felix,[6] who designated them *O* and *H* antigens respectively (p. 269). The *O* antigens are arbitrarily designated by Roman numerals. Two species of *Salmonella* also contain Vi antigen (p. 399), *S. paratyphi C* and *S. ballerup*; this antigen is heat-stable and in this and certain other respects appears to be closely related to the *O* antigens.

These two types of antigen, each of which may be, and frequently is, represented in a single bacterial strain by more than one component, differ from one another in several respects. The flagellar antigen is the more unstable and is destroyed by boiling and by exposure to alcohol or weak acid; somatic antigen, on the other hand, is stable to boiling, alcohol and acid. Cultures on phenol agar (0.1 per cent) of bacteria normally containing both *H* and *O* antigens are found to contain only *O* antigen, the formation of flagellar antigen having been suppressed; *H* antigen reappears immediately on cultivation on nutrient agar. In the agglutination reaction, bacteria lacking flagellar antigen are characteristically clumped in a finely granular precipitate (*O* agglutination), while bacteria containing flagellar antigen are agglutinated in a coarse, flocculent precipitate (*H* agglutination).

The *H* and *O* agglutinin titer of an antiserum may be determined by the use of *H* and *O* antigens in the agglutination test. *H* antigen is commonly prepared by adding an equal volume of formol (0.6 per cent formalin) saline to an eighteen- to twenty-four-hour broth culture. In the preparation of somatic antigen the flagellar component is destroyed by treatment with alcohol; the growth from an eighteen- to twenty-four-hour agar slant culture is emulsified in 1 to 2 cc. of absolute alcohol, heated at 60° C. for one hour, centrifuged and the sediment suspended in 0.5 to 1 cc. saline. It may be used for slide agglutination or appropriately diluted for macroscopic agglutinin titrations.

These two types of antigen are immunologically independent, and the immunization of an animal with a microorganism containing both results in the production of antibodies to both. There is, however, a marked difference in titer, for the *O* antibody titer is generally much lower than that of the *H* antibody; in the writer's laboratory antisera having *H* titers of 1:20,000 to 1:50,000 have shown *O* titers of 1:2000 or less.

Specific and Non-specific Flagellar Antigens.—The flagellar antigen is, in turn, of dual nature. One kind, designated as *specific flagellar antigen*, is individualistic and contributes in no small part to the immunological identity of a given *Salmonella* species. The other, termed *non-specific flagellar antigen*, is made up of a limited number of components which are frequently shared by the various *Salmonellas* and hence contribute to the immunological relationship between the species of these bacteria. The specific flagellar antigens are arbitrarily designated by lower case letters (the choice was unfortunate since the more recently discovered antigens are designated z_1, z_2, z_3, etc.), and the non-specific ones by Arabic numerals.

[6] Weil and Felix: Ztschr. f. Immunitätsforsch., 1920, *29*:24.

THE SALMONELLA TYPES (SPECIES)

Immunologic Types		Antigenic Formulae*			
		Somatic Antigens		Flagellar Antigens	
Group	Species			Phase 1	Phase 2
A	*S. paratyphi A*	(I), II, XII		a	
	S. paratyphi B	(I), IV, (V)		b	(1, 2)
	S. abony	(I), IV, V		b	e, n, x
	S. typhi-murium	(I), IV, (V)		(i)	1, 2, 3
	S. stanley	IV, V		d	1,2
	S. heidelberg	IV, V		r	1, 2, 3
	S. chester	IV, (V)		e, h	e, n, x
	S. san diego	IV, (V)		e, h	e, n, z_{15}
	S. salinatis	IV		d, e, h	d, e, n, z_{15}
	S. saint paul	I, IV, V		e, h	1, 2, 3,
	S. zagreb	IV, V		e, h	1, 2
	S. reading	IV		e, h	1, 5
	S. kaposvar	IV, V		e, (h)	1, 5
	S. koeln	IV, V		y	1, 2
B	*S. kaapstad*	IV	XII	e, h	1, 7
	S. derby	(I), IV		f, g	
	S. essen	IV		g, m	
	S. budapest	I, IV		g, t	
	S. california	IV		g, m, t	
	S. brandenburg	IV		l, v	e, n, z_{15}
	S. bispebjerg	I, IV		a	e, n, x
	S. abortus equi	IV			e, n, x
	S. arechavaleta	IV, (V)		a	1, 7
	S. abortus ovis	IV		c	1, 6
	S. altendorf	IV		c	1, 7
	S. abortus bovis	(I), IV, XXVII		b	e, n, x
	S. bredeney	I, IV, (XXVII)		l, v	1, 7
	S. schleissheim	IV, XXVII		b, z_{12}	
	S. paratyphi C	VI, VII, (Vi)		c	1, 5
	S. cholerae-suis	VI, VII		(c)	1, 5
	S. typhi-suis	VI, VII		(c)	1, 5
	S. thompson	VI, VII		(k)	1, 5
	S. montevideo	VI, VII		g, m, s	
	S. oranienburg	VI, VII		m, t	
	S. virchow	VI, VII		r	1, 2, 3
	S. oslo	VI, VII		a	e, n, x
C-1	*S. amersfoort*	VI, VII		d	e, n, x
	S. braenderup	VI, VII		e, h	e, n, z_{15}
	S. potsdam	VI, VII		l, v	e, n, z_{15}
	S. bareilly	VI, VII		y	1, 5
	S. hartford	VI, VII		y	e, n, x
	S. mikawashima	VI, VII		y	e, n, z_{15}
	S. tennessee	VI, VII		z_{29}	
	S. infantis	VI, VII		r	1, 5
	S. concord	VI, VII		l, v	1, 2, 3
	S. georgia	VI, VII		b	e, n, z_{15}

* Naturally occurring diagnostic formulae; experimental phase alterations not taken into account.

Legend: ( ) — antigen may be absent; [ ] — incomplete antigen.

THE SALMONELLA TYPES (SPECIES)—*Continued*

Immunologic Types		Antigenic Formulae*		
		Somatic Antigens	Flagellar Antigens	
Group	Species		Phase 1	Phase 2
C-2	*S. newport*	VI, VIII	(e, h)	1, 2, 3
	S. kottbus	VI, VIII	e, h	1, 5
	S. muenchen	VI, VIII	d	1, 2
	S. mexicana	VI, VIII	d	1, 2, 4
	S. pueris	VI, VIII	e, h	1, 2
	S. oregon	VI, VIII	d	1, 2, 3
	S. manhattan	VI, VIII	d	1, 5
	S. litchfield	VI, VIII	l, v	1, 2, 3
	S. morbificans bovis	VI, VIII	r	1, 5
	S. narashino	VI, VIII	a	e, n, x
	S. bonariensis	VI, VIII	i	e, n, x
	S. glostrup	VI, VIII	z_{10}	e, n, z_{15}
	S. duesseldorf	VI, VIII	z_4, z_{24}	
D	*S. typhi*	IX, (Vi)	d	
	S. enteritidis	(I), IX	g, m	
	S. dublin	I, IX	g, p	
	S. rostock	I, IX	g, p, u	
	S. moscow	IX	g, q	
	S. blegdam	IX	g, m, q	
	S. berta	IX	f, g, t	
	S. sendai	(I), IX	a	1, 5
	S. loma-linda	IX } XII	a	e, n, x
	S. durban	IX	a	e, n, z_{15}
	S. onarimon	I, IX	b	1, 2
	S. eastbourne	(I), IX	e, h	1, 5
	S. panama	I, IX	l, v	1, 5
	S. dar-es-salaam	I, IX	l, w	e, n
	S. goettingen	IX	l, v	e, n, z_{15}
	S. javiana	(I), IX	l, z_{28}	1, 5
	S. gallinarum	IX		
E-1	*S. london* (L II)	III, X, XXVI	l, v	1, 6
	S. give	III, X, XXVI	l, v	1, 7
	S. uganda	III, X, XXVI	l, z_{13}	1, 5
	S. anatum	III, X, XXVI	e, h	1, 6
	S. muenster	III, X, XXVI	e, h	1, 5
	S. nyborg	[III, X, XXVI]	e, h	1, 7
	S. vejle	III, X, XXVI	e, h	1, 2, 3
	S. maleagridis	[III, X, XXVI]	e, h	l, w
	S. shangani	III, X, XXVI	d	1, 5
	S. zanzibar	[III, X, XXVI]	k	1, 5
	S. amager	III, X, XXVI	y	1, 2, 3
	S. lexington	[III, X, XXVI]	z_{10}	1, 5
	S. weltevreden	III, X, XXVI	r	z_6
E-2	*S. newington*	III, XV	e, h	1, 6
	S. selandia	III, XV	e, h	1, 7
	S. new brunswick	III, XV	l, v	1, 7
	S. illinois	[III], [XV], XXXIV	z_{10}	1, 5
E-3	*S. senftenberg*	I, III, XIX	g, s, t	
	S. niloese	I, III, XIX	d	z_6
	S. simsbury	I, III, XIX	z_{27}	
	S. taksony	I, III, XIX	i	z_6

THE SALMONELLA TYPES (SPECIES)—*Continued*

Immunologic Types		Antigenic Formulae*		
		Somatic Antigens	Flagellar Antigens	
Group	Species		Phase 1	Phase 2
F	*S. heves*	VI, XIV, XXIV	d	1, 5
	S. carrau	VI, XIV, XXIV	y	1, 7
	S. onderstepoort	[I], VI, XIV, XXV	e, [h]	1, 5
	S. florida	[I[, VI, XIV, XXV	d	1, 7
	S. madelia	[I], VI, XIV, XXV	y	1, 7
	S. amherstiana	[VIII]	l, [v]	1, 6
	S. kentucky	[VIII], XX	i	z_6
	S. aberdeen	XI	i	1, 2, 3
	S. rubislav	XI	r	e, n, x
	S. pretoria	XI	k	1, 2, 3
	S. solt	XI	y	1, 5
	S. grumpensis	XIII, XXII	d	1, 7
	S. poona	XIII, XXII	z	1, 6
	S. borbeck	XIII, XXII	l, v	1, 6
	S. mississippi	I, XIII, XXIII	b	1, 5
	S. wichita	I, XIII, XXIII	d	
	S. havana	I, XIII, XXIII	f, g	
	S. worthington	I, XIII, XXIII	l, w	z
	S. hvittingfoss	XVI	b	e, n, x
	S. gaminara	XVI	d	1, 7
	S. szentes	XVI	k	1, 2, 3
	S. kirkee	XVII	b	1, 2
	S. cerro	XVIII	z_4, z_{23}, z_{25}	
	S. minnesota	XXI, XXVI	b	e, n, x
	S. tel aviv	XXVIII	y	e, n, z_{15}
	S. ballerup	XXIX, (Vi)	z_{14}	
	S. urbana	XXX	b	e, n, x

The antibodies to these antigens behave independently; in the presence of homologous *H* and *O* antibodies heated antigen agglutinates in the characteristic fine granular form, while with unheated bacilli coarse flocculation occurs, mixed, within the range of the *O* antibody titer, with the granular clumps. The antibodies may be selectively absorbed by appropriate antigens, and such absorbed sera are sometimes designated *monospecific sera.* By the absorption technique using homologous, heterologous or treated antigens, the antigenic components of the bacterial cell may be determined, and through manipulations of this kind, termed *antigenic analysis* (p. 268), White,[4] Kauffmann[7] and others have elucidated the complex antigenic structure and interrelationships of the bacteria of the *Salmonella* group.

Antigenic Formulae.—By identifying these antigenic components it becomes possible to write a formula that describes the immunological character of a given species or strain. Such formulae for antigenic structures are commonly used and are illustrated in the accompanying table.

[7] *Cf.* Kauffmann: Ztschr. f. Hyg. u. Infektionskr., 1937, *120:*177.

Classification.—While the bacteria given the common generic name *Salmonella* are obviously closely related to the other members of the enteric group, the interrelationships of the varieties or types of these bacilli to one another is not altogether clear. As indicated above, prior to the emphasis upon immunological character which has been marked since the 1920's, bacteria of this group were separated from one another on the basis of physiological characters. In the more commonly occurring varieties, at least, there is a marked association between biochemical properties and immunological constitution. It would seem neither justified nor desirable to subordinate completely the physiology of these bacteria to their antigenic character.

However this may be, the extent to which antigenic composition should be allowed to determine *Salmonella* species is open to question. At first it was customary to consider a new immunologic type as a new species, and many of these were given place names. As a consequence a great number of species have been described and continue to be, at the rate of several every year, and at the present time the situation has become absurd. Simplification is obviously essential, but what form it will take is as yet not clear.

Variation.—Possibly because the antigenic structure of the *Salmonella* group is known in considerable detail, two general types of variation may be differentiated. One of these is a type of fluctuating, completely reversible immunological variation known as phase variation, and the other the S-R dissociation known to occur in practically all bacteria.

Phase Variation.—The *H* antigens of certain *Salmonella* types can be separated into relatively stable components. This was first demonstrated by Andrewes[8] and may be shown very simply by plating out such a type and carrying out slide agglutination of individual colonies with monospecific antiserum. About half the colonies will be found to contain one kind of antigen and about half the other. Apparently the individual bacterial cells do not contain both kinds of *H* antigen but are of two kinds in this respect.[9] This immunologic character breeds true to only a limited extent, for if a colony is picked and subcultured in broth, platings of successive broth cultures will show a rapid reversion to the 50:50 ratio within a very few transfers. This type of immunological variation is called *phase variation* and the transitory immunological types were originally called the *specific phase*, *i. e.*, that characterized by the presence of specific flagellar antigens, and the *non-specific phase*, *i. e.*, that in which the non-specific antigens occurred. This division is not as sharp as once thought, however, and now these phases are commonly referred to as *phase 1* and *phase 2* respectively.

The *Salmonella* types that exist in two immunological phases are termed *diphasic*, while those that exist in only one phase, which may be either phase 1 or phase 2, are called *monophasic*. There is reason to believe that the monophasic types are potentially diphasic, and possibly they are degenerated diphasic forms, for it has been shown[10] that *S. paratyphi A*, a mono-

[8] Andrewes: Jour. Path. Bact., 1922, *25*:505.
[9] *Cf.* the studies of Archer: Jour. Roy. Army Med. Corps, 1941, *77*:188.
[10] Bruner and Edwards: Jour. Bact., 1941, *42*:467.

phasic type stable in phase 1, can be induced to form phase 2 antigens by cultivation in antiserum specific to the phase 1 antigens. Conversely, diphasic types can be stabilized in one phase by cultivation in the presence of antiserum to the antigens of the other phase.[11] Phase variation does not occur, of course, in *S. pùllorum* which is non-motile and contains no flagellar antigen.

Phase variation is somewhat more complex than this, however, and three types have been described. The first is the specific-non-specific phase variation of Andrewes[8] in which a specific antigen ordinarily occurring in phase 1 is linked with the non-specific antigens of phase 2. The second is the so-called α-β phase variation described by Kauffmann and Mitsui[12] in which an antigen occurring in phase 1 is linked with the antigens e, n, +, in phase 2. Five types of α-β phase variation have been reported by Edwards and Bruner[13]. Lastly, there is a type of phase variation which has not been named in which the antigens of both phases are commonly found in phase 1.[14]

It is not clear whether this type of immunological variation occurs generally with respect to the somatic antigens. A similar variation does occur, however. Kauffmann[15] has found that of the three components of XII, designated XII_1, XII_2, and XII_3, XII_2 varies in that it is either strongly or weakly developed. Antigen VI is likewise subdivided into VI_1 and VI_2 but similar variation in these components has not as yet been reported.

It is to be emphasized that the immunological changes associated with phase variation are, so far as is known, normal, the bacterial strain seemingly existing in a sort of immunological equilibrium. These variations and the complete but temporary loss of flagellar antigen by cultivation on phenol agar noted above apparently bear no relation to the S-R dissociation though, as indicated earlier, they may be induced by a method which will induce dissociation, *i.e.*, cultivation in specific antiserum. The phylogenetic implications of the pattern of distribution of antigens within the *Salmonella* group and of phase variation have been discussed by White,[4] Bruner and Edwards,[13] and Kauffmann[15] but cannot be considered here.

Dissociation.—S-R dissociation, similar in all respects to that known in other bacteria, occurs in cultures of these bacilli. The dissociation from smooth to rough is manifested as an alteration in colonial morphology and loss of virulence. The change is reflected immunologically as a loss of specificity of the somatic antigens, *i.e.*, the rough forms remain motile. The specificity of these antigens is apparently determined by a polysaccharide haptene, and with the disappearance of the haptene the bacteria acquire a new and common immunologic character in the somatic antigens, while the flagellar antigens remain unchanged. A mucoid or M phase in colonial morphology has been reported by some workers which is said to be associated with the development of a new immunological specificity.

[11] Bruner and Edwards: Kentucky Agr. Expt. Station Bull., 1940, *404:*289.
[12] Kauffmann and Mitsui: Ztschr. f. Hyg. u. Infektionskr., 1930, *111:*740.
[13] Edwards and Bruner: Jour. Inf. Dis., 1941, *69:*220.
[14] Edwards and Bruner: Jour. Hyg., 1938, *38:*716.
[15] Kauffmann: Jour. Bact., 1941, *41:*127.

Bacteriological Diagnosis of Salmonella Infection.—The differentiation of the paratyphoid fevers from typhoid fever and the determination of the etiology of gastro-enteritis caused by *Salmonella* is necessarily dependent on the isolation and identification of the causative microorganism.[16] For isolation both enrichment culture and direct plating should be used; enrichment broth and differential selective agar plates should be inoculated simultaneously and, if the latter are negative, fresh plates can be inoculated from the enrichment culture. It is commonly observed that no single agar medium suffices, for with very few bacteria cultures may be isolated on one medium but not the others; at least two, and better three, kinds of differential agar should be used.

DIFFERENTIAL REACTIONS OF SALMONELLA SPECIES

Species	Xylose	Trehalose	Arabinose	Dulcitol	Inosite	H_2S	Tartrate
S. paratyphi A	−	+	+	+*	−	−	alk
S. paratyphi B	+	+	+	+	±	+	alk
Suipestifer group:							
Para C (Hirschfeld)	+	+	+	+	−	+	
Kunzendorf (European)	+	−	−	−	−	+	
Cholerae-suis (American)	+	−	−	−	−	−	acid
S. typhi-murium (*aertrycke*)	+	+	+	+	±	+	acid
S. enteritidis	+	+	+	+	±	+	acid

* Usually delayed.

Two enrichment media are commonly used. Selenite-F broth contains 0.4 per cent sodium acid selenite, which is toxic for all gram-negative bacteria but is detoxified sufficiently in the presence of 1 per cent sodium phosphate to permit rapid growth of enteric pathogens while temporarily (eight to twelve hours) inhibiting *Bact. coli.* Tetrathionate broth contains thiosulfate, tetrathionate and iodide, the tetrathionate being formed by oxidation of the thiosulfate with iodine. The differential selective agars used contain lactose and an indicator (often neutral red) together with bile or bile salts. Of these the most commonly used are desoxycholate-citrate agar (D-C), Shigella-Salmonella agar (S-S), and MacConkey agar. The bismuth-sulfite medium of Wilson and Blair and eosine-methylene blue agar (EMB) are more useful for typhoid and dysentery bacilli respectively but are often inoculated also. On the first three the non-lactose-fermenting bacteria form colorless opaque or translucent colonies which are readily differentiated from the red colonies of the lactose-fermenters.

Typical colonies are picked and subcultured in dextrose, lactose and sucrose sugar broths, tryptophane broth, litmus milk and buffered peptone water (for the Voges-Proskauer test), and gram-stained smears are examined. Positive lactose (and/or sucrose) fermentation, positive Voges-Proskauer test, or the formation of indol excludes *Salmonella.* Litmus milk should become alkaline in four to seven days. Lactose broth cultures should

[16] Isolation procedures are discussed in some detail by Littman: War Med., 1943, *4*:31

be retained for not less than two weeks to eliminate slow lactose fermentation. Morphologically typical bacilli giving characteristic reactions in these media may be provisionally identified as pathogenic enteric bacilli. Biochemical differentiation may be carried further with additional sugar broths, tests for gelatin liquefaction, hydrogen sulfide formation, and the use of special media such as double sugar agar, tartrate medium, etc.

Salmonella Typing.—With the definition of *Salmonella* types as immunological types, complete identification requires the demonstration of at least part of the antigenic structure of the unknown bacterium. This is readily accomplished by slide agglutination with monospecific antisera. If the serological identification includes the rare types, a very considerable number of antisera are required. According to Bornstein,[17] however, more than 95 per cent of the strains encountered in this country can be identified with relatively few antisera. These include six *O* antisera: II; IV, V; VI, VII; VI, VIII; IX; III, X, XXVI; eleven monospecific *H* antisera: a; b; i; e, h; f, g; m, t; y; g, m, s; d; l, v; 1, 2; and a Vi antiserum. The dilution of the antiserum used in slide agglutination depends upon its titer; in the writer's laboratory 1/100 of the macroscopic titer gives rapid and reliable slide agglutination. *Salmonella* typing has very considerable epidemiological value and is becoming more and more generally used.

Ecology.—The distribution of *Salmonella* types in man and higher animals has been a matter of considerable interest in recent years with the more general application of serological typing. Aside from *S. paratyphi A*, which appears to be almost exclusively a human pathogen, and *S. paratyphi B*, which is found only occasionally in animals, these bacteria have been regarded as primarily parasites of birds and others animals; some, such as *S. pullorum*, produce a characteristic symptom complex. It has become increasingly clear, however, that very many of the *Salmonella* types are found in man associated with pathological conditions, and it seems probable that transmission from animals to man and from man to man occurs with some frequency. Edwards and Bruner[18] have reported that of 2520 cultures of *Salmonella* (exclusive of *S. pullorum* and *S. gallinarum*), 1258 were isolated from fowls, 532 from man, 475 from swine, 90 from rodents, 88 from carnivores, 53 from horses, and 20 from ruminants. The cultures from human sources included 41 types of *Salmonella*; of those occurring more frequently 132 were *S. paratyphi B*, 60 were *S. typhimurium*, 53 were *S. newport*, 37 were *S. newington*, 28 were *S. cholerae-suis* var. *Kunzendorf*, 27 were *S. panama* and 24 were *S. montevideo*. If these results are in any way representative, as is highly probable, it is apparent that the *Salmonella* types are widely distributed in man as well as in animals.

Pathogenicity for Man.—Two main types of *Salmonella* infection in man may be distinguished. These are the slow continued fevers of the typhoid type, *i.e.*, the paratyphoid fevers, and the sudden, usually transient, stormy gastro-intestinal disturbances of the food poisoning type. In addition to these a wide variety of clinical diseases is occasionally associ-

[17] Bornstein: Jour. Bact., 1942, *44*:719.
[18] Edwards and Bruner: Jour. Inf. Dis., 1943, *72*:58.

ated with *Salmonella* infection, including a generalized invasion with bacteremia, bone infections and other localized infections, infections of the central nervous system and the like. The healthy carrier state in man appears to be considerably more common than has been thought and carrier transmission of infection undoubtedly occurs. Of 474 cultures isolated from human sources by Edwards and Bruner,[18] 225 were isolated from fecal specimens from cases of gastro-enteritis, 123 from the feces of healthy carriers, 86 from the feces in enteric fever and 40 by blood culture. In this series there were 12 known fatalities, 3 in the cases of gastro-enteritis

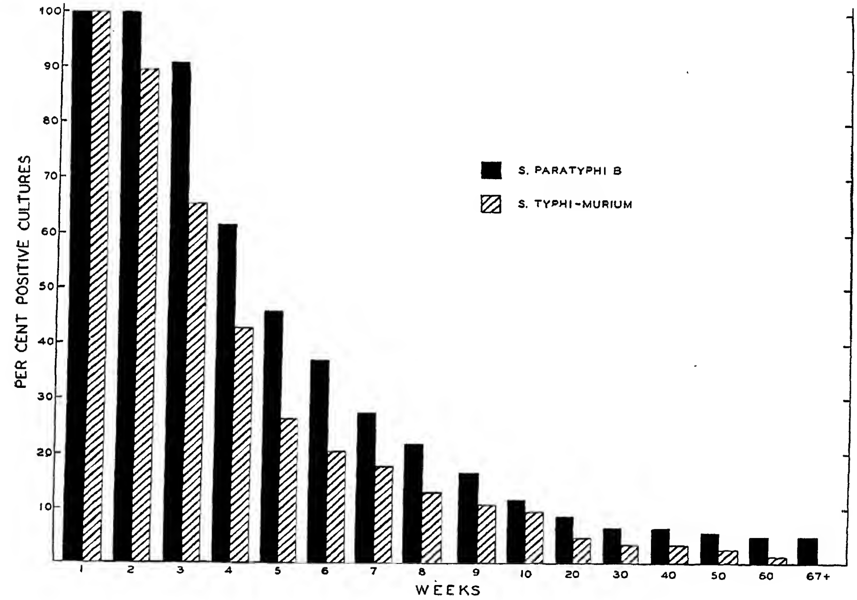


Fig. 66.—The persistence of *Salmonella* infection as indicated by positive fecal cultures by weeks. Data on 203 cases of *S. paratyphi B* infection and 239 cases of *S. typhimurium* infection by Rubenstein, Feemster and Smith: Amer. Jour. Pub. Health, 1944, *34*:841.

and 9 in those from whom positive blood cultures were obtained. Of the *Salmonella* types only the more important can be considered here.

The Paratyphoid Fevers.—Achard and Bensaude[19] in 1896 isolated a bacillus from human tissues during convalescence from a typhoid-like disease that resembled the typhoid bacillus but differed from it in important particulars. Later Gwyn[20] reported a case which apparently presented all the clinical symptoms of typhoid fever in which a bacillus closely related to *S. enteritidis* was isolated from the blood. Since that time a number of investigators in different parts of the world have isolated similar bacteria

[19] Achard and Bensaude: Soc. med. des Hop. de Paris, 1896, 3d S., *13*:679.
[20] Gwyn: Bull. Johns Hopkins Hosp., 1898, *9*:54.

from the blood of patients suffering from a disease that, so far as clinical symptoms are concerned, is substantially identical with typhoid fever.

Many cases of paratyphoid infection show a tendency to run a relatively mild course and are marked by a sudden onset with chills, but are otherwise very similar to infections with the typhoid bacillus. A certain proportion of the negative agglutination tests in apparent typhoid fever are probably a result of paratyphoid infection. The only method by which typhoid and paratyphoid fever can be distinguished is isolation and identification of the causal microorganism.

Many scattered cases of paratyphoid fever have been observed, and a number of more or less extensive epidemics have been reported as due to milk and other foods, to contact with human carriers, to sewage-polluted water and similar factors. In general, the mode of dissemination of paratyphoid fever is practically identical with that of typhoid fever (p. 405).

The frequency of the paratyphoid fevers as compared with typhoid fever varies a good deal in different localities, but most hospital records give a ratio of less than 1:10. In some regions the proportion of paratyphoid to typhoid may be as high as 1:4 or even more. During the first World War the proportion of paratyphoid to typhoid reached a high point. In the British armies in France during the years 1915–18, the diagnosed paratyphoid fevers outnumbered the typhoid cases 2:1. In civilian populations in most countries paratyphoid fevers probably amount to 5 or 10 up to 50 per cent or more of all *fully diagnosed* enteric cases.

Three different species have been commonly recognized as the cause of paratyphoid fever: *S. paratyphi A*, *S. paratyphi B* and *S. paratyphi C*. It may be noted that a mixed vaccine is commonly used in the prophylactic inoculation against typhoid fever which includes not only typhoid bacilli but *para A* and *para B* bacilli also (p. 409); *para C* is not ordinarily included.

Salmonella Paratyphi A (*Bacillus paratyphosus A*, *Bacterium paratyphosum A*, *Salmonella paratyphi*).—This bacterium differs culturally from most of the other *Salmonella* species in its inability to ferment xylose, and it is, in addition, serologically distinct. Some outbreaks due to this microorganism have been traced to sewage-contaminated water supplies, others to food contaminated through the agency of human carriers.[21] Paratyphoid fever due to *S. paratyphi A* is often very mild, 300 cases occurring in a United States infantry regiment without a single death.[22]

Salmonella Paratyphi B (*Bacillus paratyphosus B*, *Bacterium paratyphosum B*, *Salmonella schottmülleri*).—*Paratyphi B* is readily differentiated from *paratyphi A*, but has confusing cultural and serological relations with certain *Salmonella* strains of the food poisoning types. As with the preceding species, the sources and modes of transmission are similar to those of typhoid fever. The source of the bacteria is generally the human carrier, but it has been recently reported that dogs[23] have been found

[21] Jordan: Jour. Prev. Med., 1929, *3*:279.
[22] Krumwiede: Jour. Inf. Dis., 1917, *21*:141.
[23] Caspersen: Norsk Mag. f. Laegevidenskapen., 1937, *98*, Forh. Norske Med. Selskab, 138; Ztschr. f. Hyg. u. Infektionskr., 1938, *120*:611; Magnusson: Ztschr. f. Hyg. u. Infektionskr., 1938; *121*:136.

responsible for small epidemics and in another instance a cow[24] was responsible for cases of the disease. In the northern United States and in northern Europe infection with this species seems considerably more frequent than with *S. paratyphi A*.

Salmonella Paratyphi C (*S. hirschfeldii*).—*S. paratyphi C* has been found in enteric fevers in parts of Asia, Africa and southeastern Europe and has been reported as an important cause of illness and death in British Guiana. Cases of endocarditis have been found to be caused by this and other suipestifer species.[25] Although the type of disease is similar to the other enteric or intestinal forms, little is known about its epidemiology. This species is closely related biologically to the hog cholera or *cholerae-suis* strains elsewhere described.

Other species or "types" have been occasionally found: *S. barielly*, isolated from cases of mild pyrexia in India; *S. enteritidis* var. *moscow*, isolated from cases of paratyphoid fever in Russia and described by Russian bacteriologists as "Paratyphus N_2"; *S. sendai*, isolated from cases of paratyphoid fever in Japan; and *S. eastbourne*, from a paratyphoid case at Eastbourne, England. In this country *S. typhi-murium*, *S. saint paul*, *S. oranienburg*, *S. hartford*, *S. sendai*, *S. panama* and others have been found associated with enteric fevers.

Paratyphoid Gastro-enteritis.—The symptoms of this type of disease are quite different from those in paratyphoid fever and comprise a more or less violent gastro-intestinal disturbance with vomiting, diarrhea, a slight rise in temperature and usually a rapid recovery. The attack may rarely pass into a septicemic infection. The descriptions of indigenous cholera or "cholera nostras" in earlier medical writings suggest this form of illness. Outbreaks of gastro-enteritis have been usually reported in connection with the consumption of particular articles of food and are commonly referred to as "food poisoning." Food-borne infection of this type has been discussed elsewhere (p. 243) and need not be considered further here. Several species of bacteria are known to be concerned.

Salmonella Typhi-murium (*Salmonella aertrycke, Bacterium aertrycke, Bacterium typhi-murium*).—This bacterium, most commonly isolated in food poisoning outbreaks in the United States[21] and in Great Britain,[26] closely resembles *S. paratyphi B* in its cultural characteristics but can be distinguished by its ability to produce acid in tartrate medium. Before differential tests were satisfactorily worked out, *S. paratyphi B* and *S. typhi-murium* were commonly confused and both termed "para B." *S. typhi-murium* is commonly found in a variety of infections in laboratory and domestic animals and in birds; and the "*B. pestis caviae*" of some writers and Nocard's "*B. psittacosis*" are, in fact, *typhi-murium*. Most of the laboratory stock cultures labeled "Danyz virus" or "bacillus of mouse typhoid" are of the *typhi-murium* type, but some are *S. enteritidis*.

Salmonella Enteritidis (*Bacterium enteritidis*).—Although found frequently in food-poisoning outbreaks, this bacterium is less common than

[24] Rosgen and Schultze-Gahmen: Deut. med. Wchnschr., 1939, *65*:1514.
[25] Forster: Amer. Jour. Med. Sci., 1939, *197*:234; Read: Jour. Inf. Dis., 1939, *65*:263.
[26] Savage and White: Med. Res. Council Spec. Rept. Ser. No. 92, 1925.

S. typhi-murium. It closely resembles *S. typhi-murium* culturally, but is said by some to differ in that it does not ferment inosite while *typhi-murium* does. The inosite fermentation is, however, frequently not clear-cut; a lowering of *p*H may be noted but sometimes not to a sufficient degree to warrant calling the fermentation positive. In the writer's experience, the inosite fermentation is variable in both species. The two may be readily separated by serological means.

Salmonella Cholerae-Suis (*Bacterium suipestifer, Bacterium cholerae-suis*, the Hog Cholera Bacillus, American Suipestifer).—This *Salmonella* is a member of a group of closely related bacteria, called the *suipestifer group*, which also contains *S. paratyphi C* or Eastern type, as noted above, *S. cholerae-suis* var. *Kunzendorf* or European type, and the Glässer-Voldagsen type, which is comprised of two species, *S. typhi-suis* and *S. typhi-suis* var. *Voldagsen*. These species may be differentiated from one another by a combination of cultural and serological methods. *S. cholerae-suis* formerly predominated in the United States, but recent work[27] indicates that now the Kunzendorf variety is found more often. This species has been implicated in outbreaks of paratyphoid gastroenteritis, although to a much lesser extent than either *S. typhi-murium* or *S. enteritidis*. *S. typhi-suis* appears to be purely an animal (pig) pathogen, but several cases of human infection with the Kunzendorf variety have occurred in the United States. According to Eschweiler, Wahlin and Snow[28] eighty-six cases of human infection with bacteremia have been reported in this country.

Other *Salmonella* species are less commonly implicated in human infections. *S. thompson* and *S. newport*, both related to the suipestifer group immunologically, have been observed a few times and other species but once; in fact, a number of the new *Salmonella* species described in recent years have been isolated from food implicated in outbreaks of enteritis.

The distinction between the "food-poisoning" strains of paratyphoid bacilli and the strains that cause slow typhoid-like fever does not seem to be a sharp one. In rare instances acute gastro-enteritis has been traced to *S. paratyphi B*, and, while illness caused by the food-poisoning bacilli is ordinarily followed by prompt recovery, fatal cases of generalized infection with bacteremia sometimes occur. In general, however, *S. paratyphi A*, *S. paratyphi B* and *S. paratyphi C* are found in the continued fevers; *S. typhi-murium*, *S. enteritidis* and, more rarely, *S. cholerae-suis* in acute gastro-enteritis.

Pathogenicity for Lower Animals.—*Salmonella* infection of rodents is quite common; *S. typhi-murium* and *S. enteritidis* cause infections of rats and mice, and these animals may become healthy carriers of the bacilli, a point of importance in connection with the epidemiology of food-poisoning outbreaks. Preparations of "rat virus" or "Ratin" consist of these bacteria and are supposed to initiate an epidemic of disease in the rat population and hence destroy it. The use of such preparations is to be condemned, for not all the rats are killed and many of the survivors become healthy car-

[27] Bruner and Edwards: Amer. Jour. Hyg., 1939, *30:*Sec. B., 75.
[28] Eschweiler, Wahlin and Snow: Ann. Int. Med., 1944, *20:*275.

riers; there is, in addition, the danger involved in leaving preparations of virulent bacilli about the home.

Salmonella infection of the horse is also quite common. Infectious abortion of mares is caused by a specific microorganism, *S. abortus equi*, which has not been found in other animals. So far as is known, man is not susceptible to infection with this bacillus. *S. typhi-murium* has also occasionally been reported in horses. Abortion in sheep has been attributed to a member of the *Salmonella* group, *S. abortus ovis*. *Salmonella* is occasionally observed in a variety of other animals.

Birds are quite commonly infected with members of the *Salmonella* group. Epidemics due to *S. typhi-murium* sometimes cause great destruction among canaries and other songbirds. Two barnyard diseases of great economic importance are due to specific *Salmonella* types: the bacillary white diarrhea of chicks caused by *S. pullorum*; and fowl typhoid caused by *S. gallinarum* (or *S. sanguinarium*). *S. pullorum* may survive in the ovaries of the fowls that recover from infection; diseased chicks may develop from the infected ova and communicate the disease to initially healthy members of the flock. Two cases of human infection with *S. pullorum* have been reported.

CHAPTER 20

THE ENTERIC BACILLI: THE TYPHOID BACILLUS

Family: *Enterobacteriaceae.* Tribe: *Salmonelleae.* Genus: *Eberthella.* Parasites. Gram-negative, non-spore-forming rods. Dextrose fermented with acid production but no gas; lactose and sucrose not fermented. Action on other carbohydrates limited and variable. Gelatin not liquefied. Indol not produced. Type species: *Eberthella typhosa.*

The infectious nature of typhoid fever was apparent in 1856 to William Budd,[1] who, on the basis of epidemiological evidence, suggested that the disease was transmitted by sewage-contaminated water and that the source of the infectious material was human feces. The typhoid bacillus, however, was discovered in 1880 by Eberth[2] in the mesenteric glands and the spleen of persons dying from typhoid fever. In 1884 Gaffky[3] succeeded in growing Eberth's bacillus on culture media. Acceptance of this microorganism as the etiologic agent of typhoid fever, however, was delayed because typhoid fever could not be reproduced in experimental animals. The immunological aspects of typhoid fever provided strong ancillary evidence, and, in the course of time, Koch's third postulate has been fulfilled by infections in man arising from laboratory accidents.

Morphology and Staining.—The typhoid bacillus closely resembles the other enteric bacteria and exhibits no distinctive morphological characters. The microorganism is a short, plump rod, ranging, as a rule, from 1 to 3.5 μ in length and from 0.5 to 0.8 μ in breadth. In smears from agar cultures the shorter forms predominate, while longer bacilli are generally observed in liquid media. It may be noted that the typhoid bacilli present in the urine of urinary carriers are frequently in the form of long filaments. This bacterium is actively motile by peritrichous flagella and generally possesses a greater number of flagella (12 to 14) than the colon bacillus (6 to 10). Spores are not formed, and the cell inclusions taken by Gaffky and others to be spores were probably either vacuoles or metachromatic granules. Capsules are not formed.

Upon agar and gelatin media the colonies of the typhoid bacillus closely resemble those of the colon bacillus and are equally variable in appearance. The "maple-leaf" appearance, arising from the irregularly notched margins and often termed typical, is by no means always seen, and the colonies are frequently round, smooth, bluish white, translucent and slightly raised. The true typhoid bacillus is never pigmented; although there have been a number of reports[4] of the isolation of yellow pigment-forming varieties,

[1] Budd: Lancet, 1856, *ii:*4.

[2] Eberth: Archiv f. path. Anat., 1881, *83:*486.

[3] Gaffky: Mitt. a. d. k. Gesund., 1884, *2:*372.

[4] Grossmann: Centralbl. f. Bakt., I, Orig., 1933, *129:*508; Castro: Deut. med. Wchnschr., 1934, *60:*1014; Dresel and Herbert: Arch. Hyg. u. Bakt., 1938, *120:*286; Rotenburg: Ztschr. Mikrobiol. Epidemiol. Immunitätsforsch. (U. S. S. R.), 1939, No. 5, 61.

these are not true typhoid bacilli.[5] Typical cultures of the typhoid bacillus grow upon the surface of acid potato, but the growth is thin, moist and colorless, and forms the so-called "invisible film" which is strikingly unlike the profuse brownish growth of the typical colon bacillus. On pieces of potato with an alkaline reaction, the growth is more like that of the colon bacillus. No great value as yet attaches to the character of growth on potato, for there is wide variation in both the reaction of potatoes and the behavior of different strains of bacilli.

The typhoid bacillus stains easily with the ordinary aniline dyes and is readily decolorized by the Gram method.

Physiology.—*Bacterium typhosum* is not nutritionally fastidious and grows readily upon the usual nutrient (beef extract) agar and gelatin. These bacteria may be grown on simple synthetic solutions containing glucose and an ammonium salt; some strains appear to require the addition of

Fig. 67.—*Bacterium typhosum*. Smear from a pure culture, Sommersby strain. Note the variation in size from coccoid to bacillary forms. Fuchsin; × 1050.

tryptophane to these solutions,[6] but it is probable that this amino acid functions as a growth stimulant rather than an essential food substance.[7] The typhoid bacillus is a facultative anaerobe, growing almost as luxuriantly under anaerobic conditions as under aerobic conditions. Some growth is apparent at 4° C. and there is no growth beyond 46° C., while the optimum temperature is 37° C. Like the other intestinal forms, the typhoid bacillus grows over a relatively wide *p*H range, 5.0 to 8.6., with an optimum at *p*H 6.8 to 7.0.

Gelatin is not liquefied and indol is not formed, but nitrates are reduced to nitrites. Hydrogen sulfide is produced. As compared to many of the other enteric forms, the typhoid bacillus is a relatively weak fermenter and it resembles *Bact. coli anaerogenes* in that acid and no gas is produced; this last

[5] Cruickshank: Jour. Hyg., 1935, *35*:354.
[6] Fildes, Gladstone and Knight: Brit. Jour. Exp. Path., 1933, *14*:189.
[7] Burrows: Jour. Inf. Dis., 1939, *64*:145; *ibid.*, 1939, *65*:134.

characteristic is of practical value in the glucose fermentation in the differentiation of the typhoid bacillus and *Salmonella* species. The acids produced in the fermentation of glucose are lactic acid for the most part, together with small amounts of ethyl alcohol, formic and acetic acids and, sometimes, succinic acid. Acetylmethylcarbinol is not formed. Although no gas may be observed in the fermentation tube, precise measurement has shown that carbon dioxide is evolved under aerobic conditions, one mol of carbon dioxide being given off for every mol of oxygen taken up.[8] Other cultural reactions of the typhoid bacillus are summarized elsewhere (p. 371), but, in general, carbohydrate fermentations, with the exception of the failure to ferment lactose, are not of great differential value.

The susceptibility of the typhoid bacillus to deleterious influences is much the same as that of the other enteric bacteria. It is killed by exposure to 55°

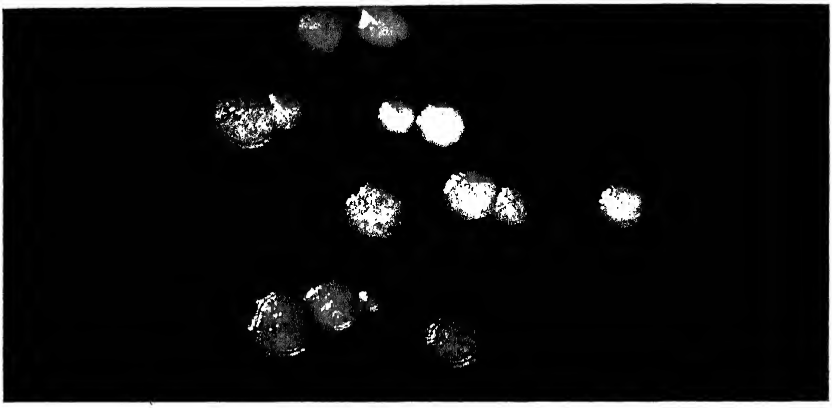


Fig. 68.—Colonies of the typhoid bacillus on nutrient agar. Note the characteristic "maple-leaf" irregular margin and slightly roughened glistening surface. × 6.

to 60° C. for thirty minutes and by the usual bactericidal chemicals in a somewhat shorter time than certain more resistant forms such as staphylococci. It persists, rather than multiplies, in nature for a variable length of time. Some bacilli remain viable in ground water for possibly two or three weeks, but in fecal matter in privy vaults and elsewhere they may persist for one to two months.[9]

Toxins.—*Bact. typhosum*, like the other enteric bacteria, does not form a soluble toxin, but its cell substance is toxic to the experimental animal upon parenteral inoculation. A polysaccharide-lipid complex which is reported to represent the somatic antigen and endotoxin has been isolated from typhoid bacilli by Boivin and Mesrobeanu[10] by trichloracetic acid

[8] As measured in Warburg respirometers in the writer's laboratory. Carbon dioxide is also given off by aerated broth cultures.

[9] Beard: Amer. Jour. Pub. Health., 1940, *30*:1077.

[10] The work on the typhoid bacillus is discussed by Boivin and Mesrobeanu: Rev. d'Immunologie, 1938, *4*:197.

extraction (p. 252). A similar substance, but differing in that it contains protein, has been isolated by Morgan and Partridge[11] by extraction with diethylene glycol and purified by precipitation from aqueous solution with acetone. The contained polysaccharide is immunologically specific, reacting with *O* antisera, and is probably the same as that studied by Freeman.[12] An alcohol-soluble substance having toxic properties has been studied by White[13] and named by him "Q" substance. Similar substances have been found in quite a number of the enteric bacilli and their relationship to the lipid complexes is not clear. Morgan[14] has studied the pathologic changes produced by the endotoxin in some detail. Intradermal inoculation results in a local edema and erythema followed by necrosis, and intravenous or intracardial injection results in congestion, hemorrhagic extravasation and necrosis in various organs. The liver and bone marrow pathology is very similar to that observed in fatal human cases of typhoid fever. The vascular epithelium is injured also and thrombosis, common in severe human infections, is produced.

Classification and Antigenic Structure.—The typhoid bacillus has been given a variety of names, including *Bacillus typhi*, *Bacillus typhosus* and *Bacterium typhosum*. Closely related to the other enteric bacilli, it is now classified by Bergey[15] as the type species of the genus *Eberthella*, one of three genera in the tribe *Salmonelleae*. The other two are *Salmonella* and *Shigella*—the latter the dysentery bacilli. Formerly termed *Eberthella typhi*, the typhoid bacillus is now designated *Eberthella typhosa*.

The antigenic structure of the typhoid bacillus is given by the formula IX, XII: d:—: and its close immunological relation to the *Salmonellas* is obvious. As a consequence of this relationship, many workers, the English in particular, allocate the typhoid bacillus to this genus and term it *Salmonella typhi*. At the present time, however, it seems best to set the typhoid bacillus apart from the paratyphoid bacilli but to use, instead of *Eberthella typhosa*, the non-committal term *Bacterium typhosum*.

Vi Antigen.[16]—In addition to the usual somatic and flagellar antigens, the majority of strains of typhoid bacilli isolated from human infections, some of which are inagglutinable by *O* antiserum, were found by Felix and Pitt[17] to contain an additional antigen. This was designated the Vi or "virulence" antigen since they believed it was associated with the virulence of the bacteria. This antigen appears to be similar to the somatic antigens, has in fact been reported[18] to be extracted with trichloracetic acid, but differs in that it is heat labile in the presence of water. It is, however, relatively heat stable in absolute alcohol, acetone or glycerine.[19] According

[11] Morgan and Partridge: Brit. Jour. Exp. Path., 1942, *23*:151.

[12] Freeman: Biochem. Jour., 1942, *36*:340.

[13] White: Jour. Path. Bact., 1932, *35*:77; Dennis: Proc. Soc. Exp. Biol. Med., 1939, *42*:89.

[14] Morgan: Amer. Jour. Path., 1943, *19*:135.

[15] Bergey: *Manual of Determinative Bacteriology*. Williams & Wilkins Company. Baltimore. 5th ed. 1939.

[16] See the review by Almon: Bact. Rev., 1943, *7*:43.

[17] Felix and Pitt: Lancet, 1934, *ii*:186.

[18] Boivin and Mesrobeanu: Compt. Rend. Soc. Biol., 1938, *128*:5.

[19] Peluffo: Proc. Soc. Exp. Biol. Med., 1941, *48*:340.

to Pijper[20] the type of agglutination produced by the antibody to this antigen differs from the normal *H* and *O* agglutination in that a sort of "paresis" is produced in the flagella, resulting in erratic movements and chance contacts between the cells. Though there appears to be no mutual attraction, the bacilli seem to be sticky and, when relatively large areas come in contact, they adhere to form clumps in which the bacilli tend to be arranged side by side.

Practically all strains of typhoid bacilli isolated from cases of the disease contain Vi antigen, though exception has been noted,[21] and more than 95 per cent of strains isolated from carriers contain it. The antigen tends to be lost as the strain is carried on laboratory media and it eventually disappears. A series of steps is apparent in this degradation. The strain is at first inagglutinable by *O* antiserum and the first step is the acquisition of *O* agglutinability, often after only one or two transfers. Next the strain loses the ability to stimulate the formation of Vi antibody, then agglutinability with monospecific Vi antiserum disappears, and finally ability to absorb Vi agglutinins from antiserum is lost. Kauffmann[22] has suggested a terminology for these changes which has been generally adopted. A strain inagglutinable in *O* antiserum is termed a *V strain*, when it agglutinates in *O* antiserum but retains other Vi characteristics it is a *V-W strain*, and when a strain gives no evidence of the presence of Vi antigen it is designated a *W strain*.

The association of *Vi* antigen and virulence is not clear-cut, and some workers have expressed doubts of its validity.[23] Virulence is difficult to measure, particularly since typhoid fever is not reproduced in laboratory animals. It may be noted that the same antigen has been found in *S. paratyphi C*, and its presence in these bacteria is not correlated with their virulence (in terms of minimum lethal dose) for mice, their natural host.[24] Nevertheless, Vi antibody has powerful protective properties as assayed by the mouse protection test and appears to be somewhat more efficient in this respect, though qualitatively no different, than *O* antibody. Antibody to Vi antigen from bacteria other than the typhoid bacillus, such as *S. ballerup*, protects mice against typhoid bacilli as well as antibody to the homologous antigen.[25]

Bacteriophage Typing.—It was shown by Craigie and Yen[26] that a number of types of Vi-containing typhoid bacilli may be differentiated on the basis of susceptibility to the lytic action of races of bacteriophage. These types and subtypes are designated by letters, A, B_1, B_2, C, etc. These phage types are apparently stable. Typing is readily accomplished by inoculating a series of areas on an agar plate and, after the inoculated areas have dried, each is inoculated separately with the type phages diluted to act selectively. After incubation an area of lysis is produced by the phage

[20] Pijper: Jour. Path. Bact., 1941, *53*:431.
[21] Dolman, Kerr and Helmer: Canadian Jour. Pub. Health, 1941, *32*:113.
[22] Kauffmann: Ztschr. f. Hyg. u. Infektionskr., 1935, *116*:617.
[23] Robertson and Yu: Jour. Path. Bact., 1936, *43*:191.
[24] Kauffmann: Ztschr. f. Hyg. u. Infektionskr., 1936, *118*:318.
[25] Luippold: Science, 1944, *99*:497.
[26] Craigie and Yen: Canadian Pub. Health Jour., 1938, *29*:448, 484.

type to which the unknown strain belongs.[27] Phage typing has been of considerable value in epidemiological studies.[28]

Variation.—The dissociation of the typhoid bacillus into the usual smooth and rough colonial types is well known. The round, domed, bluish white colonies observed in blood cultures are the typical smooth form, while the rough colonies are flatter, with a roughened surface and irregular edges, and are more opaque. The rough forms are not necessarily non-motile, and in this respect four types may be distinguished: the smooth motile, the smooth non-motile, the rough motile and the rough non-motile. The S-R transformation is, as in the case of the *Salmonellas*, associated with a change in the immunological specificity of the somatic or *O* antigens. The *Vi* antigen is apparently independent and may be present either with or without *O* antigen. A series of antigenic combinations is, then, possible, for each of the four above types may or may not contain *Vi* antigen, and *H* antigen, *O* antigen and *Vi* antigen may be present separately or in any combination. The presence or absence of *Vi* antigen is not associated with the S-R transformation, and the gradual disappearance of this antigen on continued cultivation on laboratory media cannot be regarded as a dissociative change.

The S-R transformation may take place in the body, and it is a common experience to find that typhoid bacilli isolated from carriers are spontaneously agglutinable, avirulent, typically rough forms. The relation of this transformation to the continued presence of the bacilli in the healthy carrier is not clear, though possibly associated with the S-R transformation that may be brought about *in vitro* by cultivation in the presence of immune serum.

Pathogenicity for Man.—Typhoid fever (enteric fever; Gr., *Abdominal-typhus* or *typhus;* Fr., *la fièvre typhoïde*) was for long one of the most widespread and important of all bacterial diseases. In the United States in 1900, there were 35,379 reported deaths from this disease, undoubtedly a low figure, and probably some 350,000 cases of typhoid fever in a population of 76,000,000—in the course of a decade perhaps one person in every 20 to 25 contracted the disease. The prevalence of typhoid fever has greatly diminished in recent years, and a large part of this decrease has taken place in the large cities. In 1937, of 93 large cities in the United States, 27 had no typhoid deaths, 52 had a death rate of 0.1 to 1.9 per 100,000, 13 had a rate of 2.0 to 4.9 and one a rate of 6.3. In the case of 78 cities for which data are available, the rate has fallen from 20.5 in 1910 to 0.26 in 1942.[29] Typhoid still persists, however, and epidemics occur from time to time, particularly in the smaller towns and rural areas. Much the same situation prevails in other countries, such as Great Britain.[30]

The common symptoms of typhoid fever include frontal headache, lack of appetite, nosebleed, the development of rose spots on the abdomen,

[27] *Cf.* Boyd: Brit. Med. Jour., 1939, Nov. 4, 902.

[28] For example, see Helmer *et al.:* Canadian Jour. Pub. Health, 1940, *31:*433; Lazarus: Amer. Jour. Pub. Health, 1941, *31:*60; Felix: Brit. Med. Jour., 1943, *i:*435.

[29] Jour. Amer. Med. Assn., 1943, *122:*1181.

[30] Lancet, 1937, *ii:*1340.

muscular weakness and diarrhea. Sometimes considered primarily an intestinal infection, the disease is, in fact, a general invasion of the body, particularly of the lymphatic system. The intestine is often regarded as the main portal of entry of the bacilli into the body, and Coleman and Buxton[31] have advanced the view that the lymphatic tissues in the intestinal wall are first invaded and the bacilli spread through the lymphatic system. After considerable multiplication has occurred (incubation period), the bacilli overflow into the blood and bacteriolysis takes place; the endotoxins which are liberated as a result of the destruction of the bacilli produce the symptoms of typhoid fever. Other evidence suggests that the body tissues may be invaded through the tonsils and gastric mucosa, but in any case the end result is the same and typhoid fever is a general and not a localized infection.

The typhoid bacillus appears in the blood stream early in the disease, *i.e.*, after the onset of symptoms, and may be cultured within the first ten days in the majority of cases, either from a blood sample or from the clot of samples sent in to a laboratory for agglutination tests. The presence of typhoid bacilli in the blood, however, does not constitute a septicemia; in fact, probably little or no multiplication takes place. The bacilli are also present in the bone marrow early in the disease, and some have urged culture by sternal puncture to facilitate diagnosis. During and after the second week typhoid bacilli may be found with increasing frequency in the feces, and the proportion of positive blood cultures drops off. The bacilli are also excreted in the urine in perhaps 25 per cent of the cases. They may often be found early in the disease in the rose spots, not in the blood but in the lymphatic spaces.

On autopsy, the intestinal walls are usually found to be extensively ulcerated, Peyer's patches and the solitary glands of the intestine being particularly involved and containing typhoid bacilli. Perforation of the intestinal wall as a consequence of ulceration is a not uncommon occurrence. The spleen is enlarged and congested and usually contains large numbers of typhoid bacilli. In both the spleen and the liver the bacilli seen in stained sections occur in groups or masses rather sharply focalized; scattered individuals are not often found.

A variety of complications may occur. The gallbladder is not infrequently infected and cystitis sometimes occurs. Suppurative and inflammatory processes may appear in other parts of the body. The osseous system seems especially open to attack, and affections of the periosteum, the bone marrow and the joints have been traced to infection with *Bact. typhosum*. Osteomyelitis may develop as long as six or seven years after recovery from typhoid fever, indicating that the typhoid bacillus can remain in contact with human tissues for years without losing its virulence. Other parts of the body are more rarely invaded during typhoid fever, but almost any organ can be attacked occasionally. The presence of *Bact. typhosum* has been reported in a brain abscess, and the cerebral and meningeal symptoms occurring in many cases of typhoid fever are directly connected with the localization of the bacilli in the meninges—bacilli have been found in spinal fluid obtained by lumbar puncture.

[31] Coleman and Buxton: Jour. Med. Res., 1909, *21*:83.

Secondary or mixed infections, especially with the pyogenic cocci and the pneumococcus, are not at all uncommon, and sometimes result in serious complications. Mixed infections with the tubercle bacillus and the anthrax bacillus have also been observed.

Carriers.—About one-third of the individuals having typhoid fever discharge bacilli for a period of three weeks after the onset of illness and about 10 per cent for eight to ten weeks; these are known as convalescent carriers. A certain proportion continue to discharge typhoid bacilli for six months or more, and in many cases over a period of several years or throughout the whole of a long life.

The development of the carrier condition is probably dependent upon the invasion of the gallbladder in the case of the fecal carriers and of the

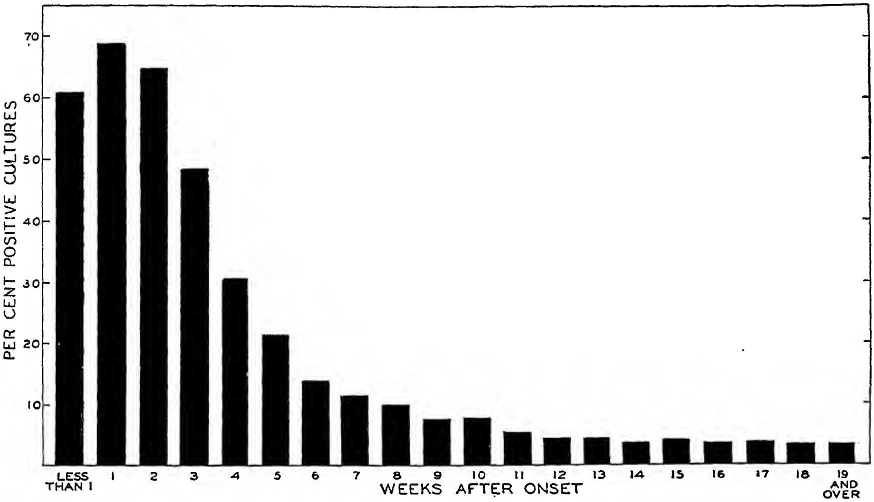


Fig. 69.—The persistence of typhoid bacillus infection as indicated by percentage of positive fecal cultures by weeks after onset. Data from 374 cases in New York State exclusive of New York City by Ames and Robins: Amer. Jour. Pub. Health., 1943, *33*:221.

urinary bladder in the case of the urinary carriers. Fecal carriers are more common than urinary carriers, and combined fecal and urinary carriers are relatively uncommon. It is not known why women are more commonly carriers than men. In the series studied by Ames and Robins[32] 2.1 per cent of the males became chronic carriers as compared with 3.8 per cent of the females. Age is a factor also; according to the same workers the percentage of cases becoming carriers was 0.3 in the 0–9 and 10–19 age groups, but as high as 10.1 in the 50–59 age group. The usual estimates for all age groups vary from 0.5 to 11.6 per cent. Typhoid bacilli need not be excreted continuously; in fact, their intermittent appearance is very common, and weeks may elapse with negative cultures before the bacilli reappear. The necessity

[32] Ames and Robins: Amer. Jour. Pub. Health, 1943, *33*:221.

for repeated examinations is, of course, obvious. A majority of carriers give the Widal reaction, and in most cases the opsonic index is abnormally high. Antibody to Vi antigen is found in the great majority of carriers, but is only transitory if present at all in inoculated persons, and the use of the Vi agglutination test proposed by Eliot[33] for the detection of carriers has given encouraging results.

Attempts to cure typhoid carriers by non-surgical means, such as chemotherapy, vaccine therapy or bacteriophage have not been generally successful, and such procedures are not generally advocated at the present time.[34] Removal of the gallbladder under suitable conditions is often effective in the case of fecal carriers; possibly three-fourths or more are cured.

The proportion of typhoid carriers in the general population is not known, owing to obvious practical and technical difficulties. It is probably quite different in different localities and doubtless depends largely upon the prevalence of current and past typhoid infection. By a modified life table procedure Ames and Robins[32] estimated a carrier rate of 42 per 100,000 in New York State. On the basis of reported deaths and assuming a case fatality rate of 10 per cent and a chronic carrier incidence of 2 per cent in survivors, Anderson, Hamblen and Smith[35] estimated a carrier rate of 48 per 100,000 in Massachusetts, and Grey[36] similarly estimated a rate of 288 in Mississippi. The number of carriers throughout the United States is probably decreasing since they are no longer produced in such quantities as when typhoid fever was prevalent. By extrapolation Ames and Robins[32] calculated that the 2500 carriers in New York State in 1940 would be reduced to 200 by 1980.

Bacteriological Diagnosis of Typhoid Bacillus Infection.—The isolation and identification of the typhoid bacillus is essential in the detection of the carrier state and is necessary to establish a diagnosis of typhoid fever. The isolation of the bacillus from blood or urine specimens is ordinarily a relatively simple matter since the fresh specimen is not heavily contaminated. Fecal specimens, however, contain very large numbers of *Bact. coli* and other bacteria and selective as well as differential media are required.

The procedure is essentially the same as that used for the isolation of *Salmonella*, and includes enrichment culture in Selenite-F broth and direct plating on S-S and D-C agars and on MacConkey agar. Blood is usually cultured in bile broth or brilliant green bile broth, though cultures in nutrient broth are often satisfactory. The bismuth-sulfite agar of Wilson and Blair[37] is particularly useful for the isolation of the typhoid bacillus for the colonies on this medium are black and distinctive; however, *S. paratyphi B* and *S. enteritidis* also grow as black colonies. The medium is strongly inhibitory and must be inoculated very heavily; consequently, colonies may not be pure cultures. Growth on this medium is often not a

[33] Eliot: Amer. Jour. Hyg., Sec. B, 1940, *31*:8.
[34] See the discussion by Cutting and Robson: Jour. Amer. Med. Assn., 1942, *118*:1447.
[35] Anderson, Hamblen and Smith: Amer. Jour. Pub. Health, 1936, *26*:396.
[36] Grey: Amer. Jour. Pub. Health, 1938, *28*:415.
[37] Wilson and Blair: Jour. Hyg., 1927, *26*:374; *ibid.*, 1931, *31*:138; *ibid.*, 1938, *38*:507

satisfactory agglutinating antigen. The medium is difficult to prepare in uniform quality but because of its utility is very widely used.

The typhoid bacillus is identified by biochemical reactions and specific agglutination and must be differentiated from other enteric bacilli which cause clinically similar disease.

Epidemiology.—The typhoid bacillus is a strict parasite found only in man. Outside the human body multiplication, if it occurs at all, is insignificant, and for practical purposes may be neglected as a factor in the dissemination of the disease. As indicated above, the typhoid bacillus leaves the body in the feces or, less commonly, the urine, and enters the body of a new host via the alimentary tract. The epidemiology of typhoid fever, then, is predicated upon the connection between the intestinal tract of the infected person and the mouth of the susceptible, and the factors that determine the spread of this disease are, essentially, those arising as a consequence of the interrelationships of the individuals or groups of individuals comprising the host population. The extent of the spread of typhoid is, of course, dependent upon the nature of the connecting links between individuals, and two epidemiological types of the disease may be distinguished, the one epidemic typhoid, and the other endemic, or residual, typhoid.

Epidemic Typhoid Fever.—Extensive outbreaks of typhoid fever necessarily involve a connecting link that is common to a great many people, and by far the most important vectors of this kind are water and milk. As pointed out elsewhere (Chapter 10), water-borne typhoid fever, formerly all too common but by now relatively rare in the larger communities, arises as a consequence of the contamination of a water supply with infectious fecal material, either as such or in the form of sewage. Water-borne epidemics of typhoid fever occur in the absence of chlorination, filtration and other purification procedures and may, of course, be readily prevented. These epidemics tend to occur in the cold months of the year, particularly in the winter and early spring, and the incidence of the disease is unaffected by age, sex or economic status.

Milk-borne typhoid fever, at the beginning of the twentieth century second only to water-borne typhoid in extent and importance, follows the route of the milkman and, as might be expected, tends to occur in the lower age groups and in families of higher economic status. The general introduction of the process of pasteurization has practically eliminated milk-borne typhoid fever from the larger urban centers, but epidemics continue to occur from time to time in various parts of the country.

Food-borne typhoid fever may take on epidemic proportions in certain instances. Oysters and other shellfish have come into bad repute in this respect in recent years, for a number of typhoid epidemics in Great Britain and the United States have been found to be due to the eating of oysters grown near sewer outfalls or placed to "fatten" in the polluted waters of estuaries or creeks. Watercress, lettuce, radishes or any vegetables or fruits which are liable to come in contact with contaminated water or are sprayed with human excrement may give rise to small-scale epidemic typhoid fever.

Endemic Typhoid Fever.—Although epidemic typhoid fever is largely eliminated in a given community through adequate sanitary control of water and milk supplies and such food supplies as are susceptible to the application of effective control measures, the disease remains in an endemic form which is manifested as occasional cases or small groups of cases which appear from time to time. The seasonal incidence is quite different from that of water-borne typhoid; the marked increase in incidence in late summer and early fall is not explained (Fig. 70). The source of infection is, of course, the case, frank or ambulatory, or the healthy carrier. Instances of direct, contact infection are unquestionably more common than is generally recognized, and the dissemination of typhoid bacilli from the infected individual to his immediate associates is undoubtedly responsible for the majority of cases of residual typhoid. Carriers are, of course, of particular importance in this connection in that they constitute semipermanent foci

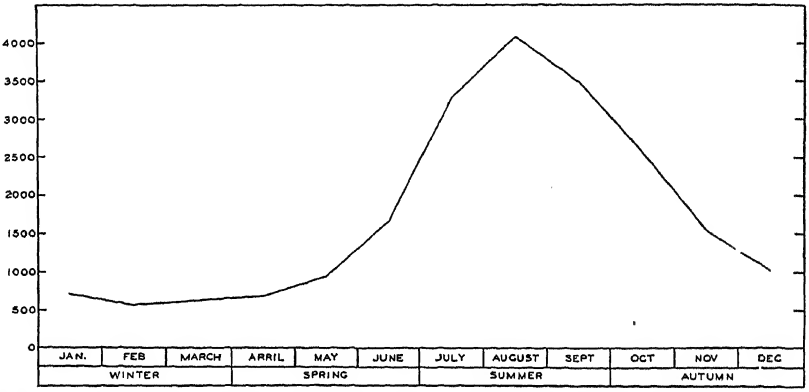


Fig. 70.—The seasonal incidence of typhoid and paratyphoid fever. Averages of reported cases by months for the years 1930 to 1938 inclusive. Data from Supplements to Public Health Reports.

of infection and, when employed as food handlers, may be the cause of small epidemics. The most notorious instance of this kind was that of Mary Mallon, "Typhoid Mary," who was unknowingly the cause of some twenty-six cases of typhoid fever in seven different families.[38] Under special circumstances, such as prevail among troops, contact infection may assume epidemic proportions.

The reduction in the prevalence of typhoid fever in the last few decades (Fig. 71) is attributable almost entirely to elimination of the great water-borne and milk-borne epidemics. As noted above, epidemics still occur, and their control is a matter of putting into practice existing knowledge. Residual typhoid, however, is much more difficult to control; the detection and supervision of all carriers, or even the elimination of carriers as food handlers, is a practical impossibility. There is reason to believe that with

[38] Soper: Jour. Amer. Med. Assn., 1907, *48*:2019.

continued control of epidemic typhoid fever, the reduction in the proportion of carriers may well be reflected in a reduced incidence of the disease in the endemic form.

Pathogenicity for Lower Animals.—The injection of typhoid bacilli into experimental animals produces much the same effect as the injection of colon bacilli. When introduced into the peritoneal cavity in considerable quantity many strains produce symptoms of a non-specific character and a fatal outcome. Although a genuine but slight multiplication of the bacilli takes place and attests the occurrence of a true infection, neither the symptoms nor the lesions of this intraperitoneal typhoid bear any close resem-

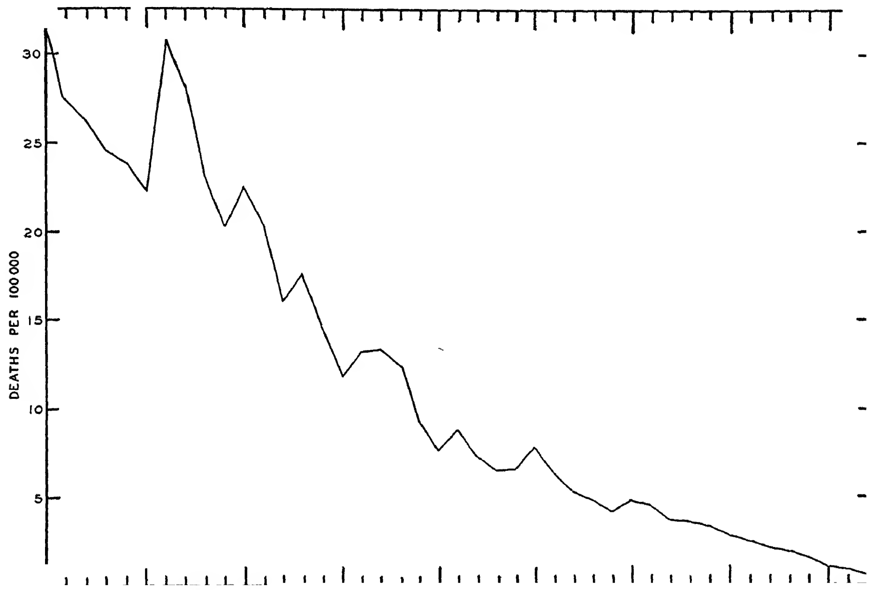


Fig. 71.—The prevalence of typhoid and paratyphoid fever in the Registration Area since 1900 as indicated by the uncorrected death rates. From data compiled by the Bureau of the Census.

blance to the typhoidal processes in man. The virulence of the typhoid bacillus for mice on intraperitoneal inoculation is raised to a very high level, only a few hundred sufficing to produce a fatal infection, by suspension in 5 per cent mucin (see Army studies below). Remlinger,[39] however, was able to set up an infection by feeding vegetables smeared with typhoid bacilli to fasting rabbits and rats, but without the reproduction of the clinical picture of human typhoid. Others have reported the reproduction of characteristic typhoid lesions in the ileum in chimpanzees fed typhoid cultures in milk and broth. It may be noted that the carrier state may be

[39] Remlinger: Ann. Inst. Pasteur. 1897, *11*:829.

produced in the rabbit by intravenous inoculation, the bacilli localizing in the gallbladder, where they may persist for several months. It must be admitted, however, that typhoid fever as it exists in man has never been reproduced in the experimental animal.

Immunity.—An attack of typhoid fever confers a certain degree of immunity, although instances of two or even more attacks in the same individual are not unknown. Animal experiment has shown that it is possible to obtain a high degree of immunity in rabbits and guinea pigs against intraperitoneal inoculation. The immunity is associated with the development of humoral antibodies such as agglutinins, precipitins and the like. Lysin is also produced, and, like the cholera vibrio, the typhoid bacillus undergoes visible dissolution and disintegration in the peritoneal cavity of the immune animal.

In man recovery from typhoid fever is also accompanied by the appearance of demonstrable antibodies. In most instances agglutinins appear during the course of the disease, sometimes as early as the fifth day (over 90 per cent by the fourth week), and their presence is the basis of the Widal test used for diagnostic purposes.

In its original form the Widal test was a slide agglutination test, and agglutination of typhoid bacilli by patient's serum in a dilution of 1:50 or more was considered positive. The development of knowledge of the antigenic structure of the typhoid and paratyphoid bacilli in recent years has resulted in a somewhat better understanding of the value and limitations of the agglutination test in the diagnosis of typhoid fever. At the present time the test is a macroscopic one and is carried out with both *H* and *O* antigens. The interpretation of a single such test must take into consideration ancillary data such as a previous immunization or attack of typhoid fever, the prevalence of endemic typhoid in the general population, etc. It is, therefore, difficult to set arbitrary limits; in most instances an *O* titer of 1:100 and an *H* titer of 1:200 may be regarded as significant. A point of some interest is the lack of sharp antigenic specificity of human sera as compared with the specificity of experimentally produced rabbit antisera.

The interpretation of the Widal test in immunized persons is often difficult, since both *H* and *O* agglutinins are formed in response to the vaccine. Titers fall after immunization, of course, but may persist at moderate levels for many months. Furthermore, the agglutinin titer may rise in anamnestic response to a febrile condition. The extent to which this occurs is not definitely known and some data suggest that it is significant while others do not. Such an anamnestic reaction is particularly prone to occur in typhus fever, and typhoid agglutinin titers as high as 1:800 have been observed.[40] There is some evidence that an "agglutinin curve," obtained by periodic agglutinin titrations, has diagnostic value in that it continues to rise in typhoid fever but usually does not do so in the anamnestic reaction.

Prophylactic Inoculation.—Man may be actively immunized against typhoid fever by the parenteral inoculation of killed typhoid bacilli. Antityphoid vaccination has had its widest application thus far in the protection of soldiers in the field, for, owing to the conditions under which they must

[40] Süpfle: Arch. f. Hyg. u. Bakt., 1943, *129*:158.

live in the field or on the march, the likelihood of typhoid fever is great. Mass immunization has effected a remarkable reduction in the incidence of typhoid fever among these men. For example, in 1898 at the time of the Spanish-American War, 4422 cases of typhoid and 248 deaths occurred in a division of 10,759 men; among 12,801 vaccinated men under very similar conditions during summer maneuvers at San Antonio, Texas, in 1911, only one case developed. Similar results have been obtained in the armies of other nations, and the efficacy of typhoid immunization is undoubted. Typhoid vaccination has been compulsory in the United States Army since 1911, and the result has been the practical disappearance of the disease.[41]

The vaccine ordinarily consists of a saline suspension of killed bacteria. The microorganisms are grown on the surface of agar culture media and after eighteen hours' incubation are washed off with sterile physiological salt solution and killed by heating to 55° to 56° C. for one hour. The suspension is standardized by counting the number of bacteria and then diluting so that 1 cc. contains 1 billion bacilli. A preservative (0.25 per cent tricresol) is added and the sterility rigidly controlled by culture and animal inoculation.

The vaccine is given in three injections, five days to a week apart. The first consists of 0.5 cc. or 500 million bacilli, and the second and third 1 cc. or 1 billion bacilli each. As a rule, the reaction following inoculation is not severe, although occasionally fever, chills, nausea and some nervous symptoms are observed. At times it has been found advantageous to immunize simultaneously against the paratyphoid infections as well. Such vaccines, known as TAB vaccines (typhoid, para A and para B), are standardized to contain 1 billion typhoid bacilli, 750 million paratyphoid A bacilli and 750 million paratyphoid B bacilli per cc. Under exceptional circumstances still other microorganisms have been added to typhoid vaccines. The Italian troops in Ethiopia, for example, experienced a considerable residue of enteric fevers after TAB inoculation. This residue was greatly reduced by the incorporation of the cholera vibrio in the standard typhoid vaccine.[42] Such procedures are, however, unusual.

In recent years this vaccination procedure has been under some criticism, particularly by Felix and his co-workers. It has been objected that heating destroys valuable antigenic components of the bacteria, that the *O* antibody is more important in the immune state than the *H* antibody but is not produced by the usual procedure, and that *Vi* antibody contributes in large measure to the immune state but that *Vi* antigen is not present in the vaccines. The significance of these criticisms is open to some question, for, contrary to earlier contentions, it has been shown that *O* antibody as well as *H* antibody is produced by immunization,[43] and, furthermore, the supposedly greater immunizing potency of *Vi* antigen as compared with *O* antigen is by no means conclusively established. While it cannot be

[41] The Army experience to 1942 with prophylactic inoculation has been summarized by Callender and Luippold: Jour. Amer. Med. Assn., 1943, *123*:391.

[42] Raffaele: Gior. Ital. di Malat. Esto. e Trop., 1936, *9*:149.

[43] See, for example, Perry: Amer. Jour. Hyg., 1937, *26*:388.

reasonably maintained that the present antigen cannot be improved, its efficacy has been proved beyond doubt by the experience of many years.

Improvement of the present antigen has been the object of intensive study at the Army Medical School in this country. Unfortunately there is as yet no wholly satisfactory means of evaluating bacterial vaccines, and questions of their efficiency as immunizing agents are particularly difficult to answer when the disease cannot be reproduced in experimental animals. The Army workers have measured immunizing efficiency in terms of the protective quality of serum from immunized animals against intraperitoneal inoculation of virulent bacilli in mucin suspension into mice. They suggest that a strain of typhoid bacilli should be used which is (1) highly virulent and (2) highly immunogenic in terms of mouse protection, these properties to be demonstrated by active and passive immunity tests in a selected breed of mice. On the basis of such mouse protection tests, the Rawlings strain (the classic strain of *Bact. typhosum*) was found to be lacking in immunizing properties, and a more virulent strain, designated as No. 58, is now used in the Army.[44]

Another type of antigen, consisting of a formolized solution of the cell substance of typhoid bacilli and designated endotoxoid vaccine, has been used in recent years with encouraging results,[45] but it is not as yet clear how this antigen compares with whole bacilli.

As indicated above, typhoid vaccines are ordinarily administered parenterally, by subcutaneous injection. It has been contended by some workers that oral administration of the vaccine together with a small quantity of bile, which presumably increases the permeability of the intestinal wall, produces an equally effective immunity. As pointed out in an earlier chapter, oral vaccination is a highly uncertain method of inoculation having no advantages over parenteral injection. Vaccination by the oral route is seldom practiced.

Passive Immunization.—The use of antityphoid serum for therapeutic purposes has been considered by a number of workers, but there is still no conclusive evidence as to its value. Whether or not "antiendotoxic" sera or other typhoid antisera confer a passive immunity to typhoid infection in man is likewise not established.

ALCALIGENES FECALIS

Alcaligenes fecalis or *Bacterium fecalis alcaligenes* closely resembles the typhoid bacillus morphologically, culturally and even in its growth on Endo, Conradi-Drigalski and malachite green differential media. It has been found in feces and in water. It differs from the typhoid bacillus in the possession of one or more polar, instead of many peritrichous, flagella; more luxuriant growth on potato with a brown coloration; and distinct alkali production in mannitol and litmus milk. It fails to produce acid from

[44] These studies are described in detail in a monograph by Siler *et al.*: *Immunization to Typhoid Fever.* Johns Hopkins Press. Baltimore. 1941. Additional studies are summarized by Longfellow and Luippold: Amer. Jour. Pub. Health, 1943, *33*:561.

[45] Grasset: Brit. Med. Jour., 1939, July 8, 58. See also Morgan, Favorite and Horneff: Jour. Immunol., 1943, *46*:301.

dextrose and other carbohydrates. It has been suggested that *B. alcaligenes* is a form of *B. fluorescens non-liquefaciens* which has lost the function of pigmentation, and it does have affinities with certain of the plant pathogens and soil bacteria. Its systematic position has been examined critically by Conn[46] who has proposed a new genus, *Agrobacterium*, to include this bacillus together with *Bacterium radicicola* and the bacterium of hairy root disease.

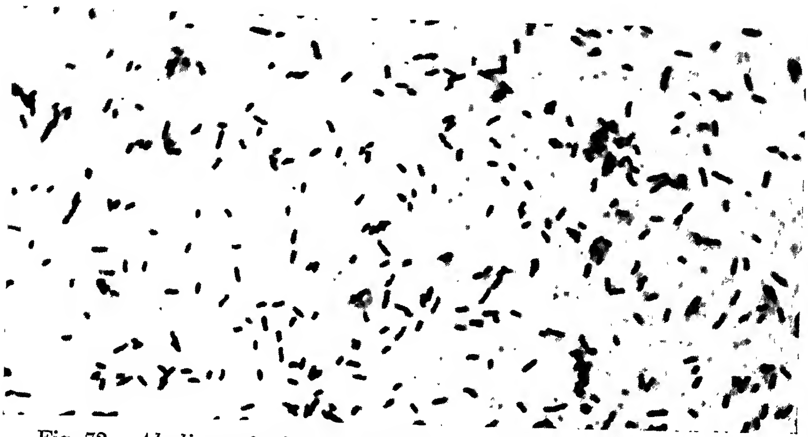


Fig. 72.—*Alcaligenes fecalis*. Smear from a pure culture. Fuchsin; × 1050.

It is only feebly pathogenic for experimental animals and presumably also for man. Human infection is observed in rare instances, however, in the form of mild typhoid-like disease, bacteremia[47] and cystitis.[48]

[46] Conn: Jour. Bact., 1942, *44*:353.
[47] Weintraub and Neter: Amer. Jour. Dis. Children, 1943, *66*:413.
[48] Ahad: Indian Med. Gaz., 1942, *77*:530.

CHAPTER 21

THE ENTERIC BACILLI: THE DYSENTERY BACILLI

Dysentery is a clinical rather than an etiological entity, and its characteristic symptoms, diarrhea, abdominal pain and blood in the stools, may occur either alone or as part of the syndrome of a number of diseases. In the former instance dysentery may be of protozoan (amebic dysentery, p. 701) or bacterial etiology. A dysentery-like infection may be produced by some members of the *Salmonella* group, but usually another group of bacteria, the dysentery bacilli, is responsible.

The dysentery bacilli are gram-negative, non-spore-forming rods related to the other enteric bacteria. Some of them resemble *Bact. coli anaerogenes* and the typhoid bacillus in that they ferment carbohydrates with the production of acid and no gas. Others are, perhaps, related to the slow or late lactose-fermenters designated as paracolon bacilli. None of the dysentery bacilli is motile, and hence they do not contain the two types of antigens found in the paratyphoid bacilli. As a group they differ from one another biochemically and immunologically. In general, their uncertain relationship to the other enteric bacilli, coupled with their own heterogeneity, has made their classification a difficult matter.

In the current Bergey classification the dysentery bacilli are members of a single genus, *Shigella*, which, together with *Eberthella* (the typhoid bacillus) and *Salmonella*, make up the tribe *Salmonelleae* in the family *Enterobacteriaceae*. Many workers, however, prefer to retain the generic name *Bacterium* for these microorganisms.

DIFFERENTIAL REACTIONS OF THE DYSENTERY BACILLI

Species	Mannitol	Lactose	Sucrose	Dulcitol	Sorbitol	Rhamnose	Indol
Bacterium shigae	−	−	−	−	−	−	−
Bacterium flexneri	+	−	−	−	+	±	±
Bacterium ambiguum	−	−	−	−	+	+	+
Bacterium alkalescens	+	−	±	+	+	−	+
Bacterium sonnei	+	+	+	−	−	+	−
Bacterium dispar	+	+	+	−	+	+	+

The dysentery bacilli are facultative anaerobes and their optimum temperature for growth is 37° C. Their nutritive requirements are not complex in that they will grow upon the ordinary nutrient (beef extract) media. In synthetic solutions nicotinic acid is apparently required by some strains, but whether amino acids are necessary is not known. They ferment glucose to much the same end products as the other enteric forms—lactic acid

together with smaller amounts of formic and acetic acids and ethyl alcohol. Like the other gram-negative bacilli, they are relatively resistant to the bacteriostatic action of dyes, and these substances may be incorporated in differential media for their isolation; eosin-methylene blue agar is commonly used.

The dysentery bacilli may be divided into several groups on the basis of fermentation reactions. The carbohydrates of particular differential value are mannitol, lactose, sucrose, dulcitol and sorbitol, and the formation of indol is useful in some instances. On this basis seven relatively well defined groups are apparent which are best considered separately.[1]

Bacterium Shigae (The Shiga Bacillus, *Bacterium dysenteriae, Shigella dysenteriae).*—This was the first of the dysentery bacilli to be described. The Japanese bacteriologist, Shiga, found the bacterium which bears his name in a study of an epidemic of dysentery in Japan in 1898.[2] The same microorganism was found by Kruse[3] in Germany two years later, and *Bacterium shigae* is known as Shiga's bacillus or the Shiga-Kruse bacillus. The Shiga bacilli are immunologically homogeneous, but anti-Shiga sera show some cross-reaction with some strains of *Bacterium flexneri* and *Bacterium ambiguum.* Strains of dysentery bacilli culturally identical with *Bacterium shigae* but immunologically unrelated were found by Dudgeon and Urquhart[4] in Macedonia and designated by them *Bacterium para-shigae* (−), in contrast to the Schmitz bacillus, which they termed *Bacterium para-shigae* (+). Strains of this bacterium have also been found in the United States.[5]

Bacterium shigae differs from the other dysentery bacilli in its marked toxicity for man and experimental animals. Apparently two types of toxin are formed; an endotoxin closely bound to the cell substance and which is a polysaccharide-lipid-polypeptide complex[6] that appears to have considerable immunizing activity as assayed by mouse protection;[7] and an exotoxin found in filtrates of broth cultures, protein in nature and thermolabile. The exotoxin (neurotoxin) has an effect upon the nervous system and produces paralysis, while the endotoxin appears to act chiefly upon the alimentary tract. Although generally regarded as a true soluble toxin, the Shiga toxin is not as potent as the toxins of the diphtheria and tetanus bacilli, nor does it stimulate the production of high titer antisera. Antitoxic sera may, however, be produced and have marked protective qualities in animal experiments. The therapeutic use of these sera has given encouraging results in some instances, but their value is not firmly established.

Shiga bacillus infections have been observed most frequently in India, Japan, China and other parts of Asia; they appear to be relatively rare in the United States.

[1] These bacilli have been reviewed by Neter: Bact. Rev., 1942, *6*:1.
[2] Shiga: Centralbl. f. Bakt., 1898, *23*:599.
[3] Kruse: Deut. med. Wchnschr., 1900, *26*:637; *ibid.*, 1901, *27*:370, 386.
[4] Dudgeon and Urquhart: Med. Res. Council Spec. Rept. Series No. 40, 1919.
[5] Hazen: Jour. Inf. Dis., 1938, *63*:330.
[6] Morgan and Partridge: Biochem. Jour., 1940, *34*:169.
[7] Schutze: Jour. Path. Bact., 1943, *55*:457.

Bacterium Flexneri (*Bacterium paradysenteriae*, Pseudodysentery bacillus, *Shigella paradysenteriae*, Flexner's Bacillus, Hiss and Russell's "Y" Bacillus, Strong's bacillus).—Soon after Shiga's discovery, Flexner,[8] working in the Philippines, discovered other dysentery bacilli which for a time were not clearly differentiated. Flexner's bacilli and those described by Strong and Musgrave[9] differed from *Bacterium shigae*, however, both serologically and in the fermentation of mannitol. Attempts to subdivide the bacilli of the Flexner group by biochemical methods have not been successful. These bacteria are immunologically heterogeneous although related to one another.

Five immunological types of Flexner bacilli were distinguished by Andrewes and Inman[10] on the basis of content of four antigens, V, W, X and Z.

Fig. 73.—*Bacterium flexneri.* Smear from a pure culture. Fuchsin; × 1050.

These types are designated V, W, X, Y and Z and their antigenic composition is illustrated in Fig. 74. A number of other systems of typing Flexner bacilli have been suggested from time to time (*cf.* Neter[1]) but none has gained the general acceptance of the Andrewes and Inman types. More recently Boyd[11] has reported the presence of group- and type-specific antigens in these and other mannitol-fermenting dysentery bacilli. According to Boyd only the V, W and Z types of Andrewes and Inman are valid since each contains a distinctive specific antigen together with a complex group antigen; Type Y is regarded as a group variant of Type W and Type X as an incomplete variant of Type Z. In addition to these, he found three other groups containing the group antigen together with type-specific antigens in a large series of Indian strains. These were designated "103," "P 119," and "88"; the last is not new but is the well-known Newcastle bacillus (see below). Boyd has suggested, therefore, that the old type designations be dropped and six types of Flexner bacilli be recognized and designated

[8] Flexner: Bull. Johns Hopkins Hosp., 1900, *11*:231.
[9] Strong and Musgrave: Jour. Amer. Med. Assn., 1900, *35*:498.
[10] Andrewes and Inman: Med. Res. Council Spec. Rept. Series No. 42, 1919.

by Roman numerals: Type I is the old Type V; Type II is the old Type W; Type III is the old Type Z; Type IV is "103"; Type V is "P 119"; and Type VI is "88" or the Newcastle bacillus. This nomenclature has been adopted by the British Army and accepted by some workers in this country,[12] but whether it will be generally adopted remains to be seen.

Bacterium flexneri does not produce an exotoxin like that of Shiga's bacillus, but its cell substance is toxic upon parenteral injection.

The Flexner bacilli are found in a large proportion of the dysentery cases in temperate countries; even in warm countries they are perhaps the most common types. In 1929 among the British and Indian troops in India Flexner bacilli were isolated in 999 cases, Shiga bacilli in 197, Schmitz bacilli in 100 and Sonne bacilli in 33. Probably many mild attacks of diarrhea are caused by Flexner bacilli. In this country 451 strains of a total of 769 isolated in routine examinations in Connecticut in 1940–43 proved to be Flexner bacilli.[12]

The Newcastle Bacillus.—The bacterium known as the Newcastle bacillus was isolated in 1929[13] from cases of dysentery. It has never been

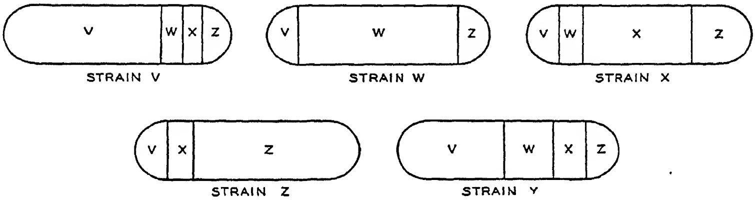


Fig. 74.—Diagrammatic representation of the antigenic structure of the five strains of the Flexner dysentery bacillus.

given a specific name and appears to be somewhat apart from the other dysentery bacilli in that it is feebly motile, ferments dulcitol and produces gas, usually a very small amount, in the fermentation of glucose. The original Newcastle bacillus did not ferment mannitol, but a variety known as the Manchester type, or Newcastle-Manchester bacillus, does ferment this alcohol; some strains produce a small amount of gas and others do not. Almost all of the Newcastle bacilli isolated in this country have been of the mannitol-fermenting, non-gas-forming type. Lactose and sucrose are not fermented and indol is not produced. According to Clayton and Warren[13] this species is immunologically homogeneous. Cross reactions with the Flexner bacilli, of which Boyd considers it a type, have been noted above. Less common than the Flexner and Sonne bacilli, the Newcastle bacillus is found in this country as elsewhere as a cause of bacillary dysentery; Hardy and his co-workers[14] reported a food-borne epidemic of 97 cases in a hospital, and Kuhns and Anderson[15] reported a fly-borne epidemic in an

[11] Boyd: Jour. Hyg., 1938, *38*:477; Trans. Roy. Soc. Trop. Med. Hyg., 1940, *33*:553.
[12] See Wheeler: Amer. Jour. Pub. Health, 1944, *34*:621.
[13] Clayton and Warren: Jour. Hyg., 1929, *28*:355; *ibid.*, 1929, *29*:191.
[14] Hardy, Frant, Jarcho and Schlosser: Pub. Health Repts., 1940, *55*:2101.
[15] Kuhns and Anderson: Amer. Jour. Pub. Health, 1944, *34*:750.

army camp involving 1557 cases. Since attention has been directed to this organism in recent years, it is likely that it will be observed more frequently than in the past.

Bacterium Ambiguum (Schmitz bacillus, *Shigella ambigua*).—First described by Schmitz[16] as the cause of dysentery in a Rumanian war prison camp, this bacillus has come to be generally regarded as constituting an authentic division of the dysentery group. It resembles the Shiga bacillus in that it does not ferment mannitol but differs in that it produces indol. This species is immunologically homogeneous and distinct from the other dysentery bacilli, although showing some cross-reactions with Shiga bacilli. It forms an endotoxin similar to those produced by the other dysentery bacilli.[17] *Bact. ambiguum* has been found in India, the Sudan and other localities, and, although rare in the United States, was implicated in an extensive institutional outbreak in New York.[18]

Fig. 75.—*Bacterium alkalescens.* Smear from a pure culture. Fuchsin; × 1050.

Bacterium Alkalescens (*Shigella alkalescens*).—*Bact. alkalescens* was described by Andrewes[19] in 1918. Unlike the other dysentery bacilli, these bacilli ferment dulcitol. For some time they have been regarded as of uncertain pathogenicity but evidence is accumulating which indicates that they are associated with disease. They have been found associated with sporadic enteric disease, and a small epidemic has been reported recently.[20] They are also pathogenic for experimental animals.[21]

Strains of *Bact. alkalescens* have been regarded as biochemically distinct and immunologically homogeneous. Stuart, Rustigan, Zimmermann and

[16] Schmitz: Ztschr. f. Hyg., 1917, *84*:449.
[17] *Cf.* Haas: Ztschr. f. Immunitätsforsch. u. Exp. Therap., 1938, *94*:480.
[18] Schliefstein and Coleman: Jour. Inf. Dis., 1937, *61*:257.
[19] Andrewes: Lancet, 1918, *i*:560.
[20] Felsen and Wolarsky: New York State Jour. Med., 1940, *40*:1303; Stuart *et al.*: Jour. Immunol., 1943, *47*:425.
[21] Edward: Jour. Path. Bact., 1940, *51*:245.

Corrigan,[22] however, have found that the species is made up of a graded series of biochemical types. They have also demonstrated the presence of five antigens, two major antigens designated A and B, and three minor antigens, C, D and E, in these bacilli; A, B and C are present in all typical strains, while D and E occur singly or in combination to give four subtypes. They are immunologically related to the colon bacilli through the paracolon group. Two immunologically unrelated types have been described[23] and designated type II and type III in distinction to the original or type I. Whether these should be classified as *Bact. alkalescens* is possibly open to some question.

Bacterium Sonnei (Sonne's Group III, Duval's bacillus, *Shigella sonnei*).—Lactose-fermenting dysentery bacilli discovered by Duval[24] in 1904 have since been rediscovered by quite a number of observers. By force of usage these bacteria have come to be known as the Sonne type. The Sonne bacillus ferments mannitol and does not produce indol; it is serologically distinctive and homogeneous. Glynn and Starkey[25] have reported, however, that there are two immunological types of *Bact. sonnei* which they designate I and II. Type I contains one antigen in predominance, while type II contains both antigens in equal amounts. The difference in agglutination titers is of practical importance and type II antiserum is the one of choice. The lactose fermentation is slow and may be delayed for a week or ten days, and strains of this type were doubtless confounded with Flexner bacilli by the earlier workers. It is probably considerably more common in the United States than appears in the records. It has also been reported in warmer countries, and perhaps a more detailed study of the differentiation of the mannite-fermenting bacilli would increase the amount of dysentery properly ascribable to this microorganism.

The slow lactose fermentation would appear to relate *Bact. sonnei* to members of the colon group and in particular the so-called paracolon bacilli, but their immunological homogeneity tends to set them off.

Bacterium Dispar (*Shigella madampensis*).—Other lactose-fermenting dysentery bacilli were designated *Bact. dispar* by Andrewes.[19] Some of Andrewes' strains were *Bact. sonnei*, but the type now termed *dispar* differs from the Sonne type in that sorbitol is not fermented and indol is not formed. *Bact. dispar* is serologically heterogeneous and not related to *Bact. sonnei* but appears to be related to certain Flexner strains. Carpenter[26] found that a group of 37 strains could be separated into three immunological types, two related to one another and the third independent. It may be noted that Bergey has set up still another species, *Shigella ceylonensis*, which differs from *Bact. dispar* in that it ferments dulcitol.

Pathogenicity for Man.—The serum of patients suffering from acute dysentery agglutinates one or another type of dysentery bacillus in high dilutions. This fact and the constant occurrence of the same type of bacillus

[22] Stuart, Rustigan, Zimmermann and Corrigan: Jour. Immunol., 1943, *47:*425.
[23] Assis: O Hospital, 1939, *15:*447, 655; Neter: Proc. Soc. Exp. Biol. Med., 1944, *57:*200.
[24] Duval: Jour. Amer. Med. Assn., 1904, *43:*381.
[25] Glynn and Starkey: Jour. Bact., 1939, *37:*315.
[26] Carpenter: Proc. Soc. Exp. Biol. Med., 1943, *53:*129.

in the stools strongly suggest a causal relation in spite of the fact that the dysentery bacilli cannot, like the typhoid bacillus, be cultivated from the blood of the patients. Laboratory infection with a pure culture of the Flexner bacillus has occurred.[27]

The incubation period of bacillary dysentery is generally short, about forty-eight hours, and the disease may be acute or tend to run a chronic course. Apart from the inflammatory, sometimes ulcerative or diphtheritic, lesions in the intestine (ulcerative colitis), the anatomical picture of dysentery presents little that is characteristic. The liver abscesses that are found, as a rule, in amebic dysentery are absent in the bacterial diseases, one series having been reported of 1130 cases of bacillary dysentery without a single abscess. Dysentery bacilli are sometimes found in immense numbers in the dejecta, often in almost pure culture. They may be found at autopsy in

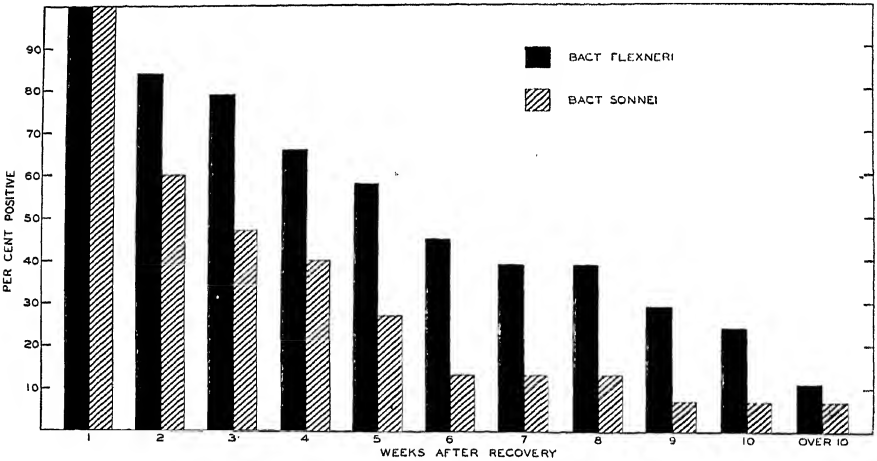


Fig. 76.—The persistence of dysentery bacilli in the feces of convalescents. Prepared from data of Watt, Hardy and DeCapito: Pub. Health Repts., 1942, *57*:524.

the mesenteric glands, but, as a rule, not in the spleen or other internal organs, nor do they commonly occur in the blood or urine. Bacillary dysentery is, therefore, not a septicemia but an infection localized in the alimentary tract, in this respect resembling Asiatic cholera rather than typhoid fever.

The dysentery toxin is excreted in rabbits, and probably in man, by the large intestine. The selective action of the toxin upon the tissues, rather than any local action of the bacilli themselves, appears to be responsible for the inflammation and other local changes. When the toxin is introduced directly into the gut, no symptoms are produced, indicating that the toxin primarily affects the deeper cells rather than the surface of the mucous membrane.

[27] Lippincott: Jour. Amer. Med. Assn., 1925, *85*:901.

In the large series of cases studied in Denmark caused by the Sonne and Flexner types the case fatality was about 2 per cent. The Shiga bacillus dysentery of the tropics is much more often fatal (20 per cent). The clinical disease is considerably more severe in the Shiga infections than in Flexner infections, and complications, such as arthritis, are almost invariably associated with the former type of infection.

The relation of dysentery bacilli to summer diarrhea of infants is obscure. Some investigators have isolated dysentery bacilli from the excreta, particularly in those cases where there is mucus in the stools. Those cases with which dysentery bacilli are associated do not appear to differ clinically from those in which they are not found, and it is uncertain just what proportion of cases of infant diarrhea is caused by the dysentery bacilli.

BIOCHEMICAL SEPARATION OF THE DYSENTERY BACILLI.

- dextrose +
  - mannitol −
    - indol +
      - *Bact. ambiguum*
    - indol −
      - *Bact. shigae*
  - mannitol +
    - lactose + (late)
      - *Bact. sonnei*
    - lactose −
      - dulcitol −
        - *Bact. flexneri*
      - dulcitol +
        - *Bact. alkalescens*

Carriers.—It is probable that dysentery bacillus infection is very common, many of the infections going unrecognized because of the mildness of the symptoms. Persons with such infections are, of course, convalescent carriers who continue to discharge the bacilli for an average of three to five weeks. The chronic carrier of dysentery bacilli does not appear to be important; possibly a condition analogous to that of the chronic typhoid bacillus carrier does not exist. In any case, it is the casual carrier and changing groups of casual carriers or ambulatory cases, as noted above, that are of primary importance in the maintenance and spread of the infection. Hardy and Watt[28] found a Shiga bacillus carrier rate (persons with no history of diarrhea in the previous year) of 3.8 per cent in New Mexico, Georgia and Puerto Rico, but only 0.1 per cent in New York City.

Bacteriological Diagnosis of Bacillary Dysentery.—Since, as indicated earlier, dysentery is a clinical rather than an etiological entity, the

[28] Hardy and Watt: Pub. Health Repts., 1942, *57*:524.
[29] Wilson and Blair: Brit. Med. Jour., 1941, *ii*:501.
[30] Thomas and Hulme: Lancet, 1942, *ii*:321.

causative microorganism must be isolated and identified to allow a diagnosis of bacillary dysentery. The bacilli may be found in fecal specimens and rectal swabs are cultured for the detection of carriers. The methods of enrichment culture and direct plating are essentially those used in the isolation of *Salmonella* and the typhoid bacillus. Desoxycholate-citrate and S-S agars are the most useful of the agar media; bismuth-sulfite is not suitable because species other than *Bact. flexneri* are inhibited. A tellurite-iron-rosolic acid medium has been developed by Wilson and Blair[29] which is said to give good results,[30] but has not as yet been generally used. Colonies are picked and identified by fermentation reactions and slide agglutination; the latter may include typing of the Flexner strains.

Epidemiology.—Infections caused by dysentery bacilli are probably far more common than is generally recognized. Attacks of severe illness grade off into mild and almost trivial attacks of simple diarrhea. Several outbreaks of typical "food poisoning" attributed to the Sonne bacillus are on record. In a number of localities where careful bacteriological studies have been made, dysentery bacilli have been found widely distributed both in patients with gastro-intestinal derangements and in the general population.

Dysenteric infections seem to be most common in hot countries and in the summer months in temperate climates, although they may occur at any season of the year. The spread of the disease is due to the more or less direct transfer of the specific bacillus from infected intestinal discharges to the alimentary tract of a fresh individual. Polluted water may play a part in some outbreaks but is apparently not nearly so important a factor in dysentery as it is in typhoid fever. Improper disposal of excreta permitting dissemination by flies, and the contamination of food by chronic carriers and convalescents appear to be the most important factors in the spread of bacillary dysentery.The rôle of insects, especially flies, is probably an important one and the seasonal incidence of bacillary dysentery is in keeping with this. In the epidemic reported by Kuhns and Anderson[15] infected flies were caught in kitchens and operating latrines; similar reports are not common. The decline in diarrhea and enteritis of the last few decades is very likely a reflection of the general improvement in sanitary conditions.

At the present time in temperate climates dysentery flourishes especially in insane asylums and other large institutions, where lack of personal hygiene among the inmates favors the transfer of infection. In the Denmark investigation endemic asylum dysentery was found to be a serious and prevalent disease. Whenever it gains a foothold in these institutions it seems to be kept alive chiefly by chronic carriers and proves an obstinate problem. Weekly bacteriological examination in one institution showed that more than 50 per cent of the dysentery patients continued to excrete dysentery bacilli for long periods—in one case over four and one-half years. Epidemic bacillary dysentery is also a disease of armies in the field, where opportunities for the dissemination of infection are frequently very great and extensive outbreaks are common.

Although in small outbreaks a single type of dysentery bacillus may be found, in the larger outbreaks more than one type is almost always ob-

served. The Shiga and Flexner types generally predominate in such outbreaks of mixed etiology, while other types are in the minority. The mannitol fermentation is, then, a differential test of great practical importance. The most common types in this country appear to be Flexner, Sonne, Schmitz and *Bact. alkalescens* in that order.

Immunity.—Antibodies, agglutinins, are formed in response to infection with dysentery bacilli, usually appearing after the sixth day. The titer is relatively low as a rule. The diagnostic significance of agglutinins is some-

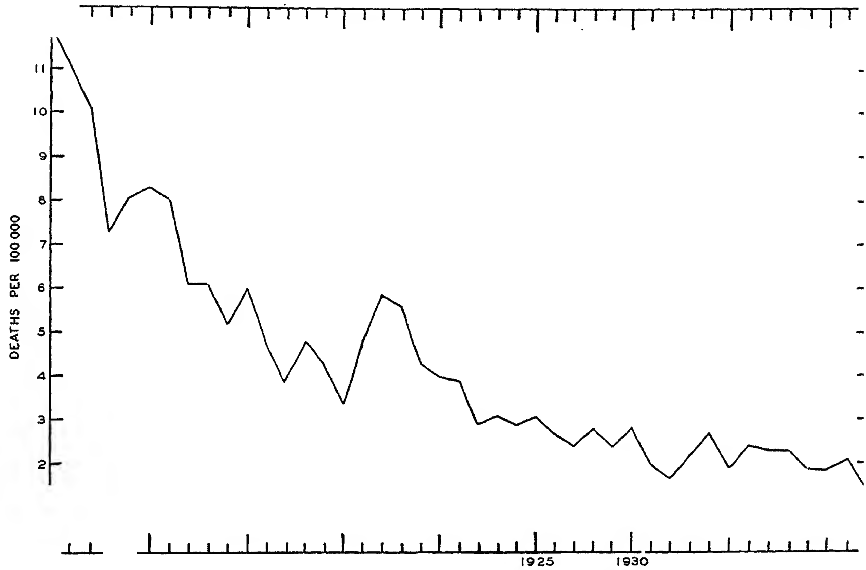


Fig. 77.—The prevalence of dysentery in the Registration Area since 1900 as indicated by the uncorrected death rates. From data compiled by the Bureau of the Census.

what uncertain largely because "normal" agglutinins are common. Normal serum commonly agglutinates *Bact. shigae* in 1:20 dilution but a titer of 1:40 or higher is suggestive of infection. Agglutinins for *Bact. flexneri* occur in much higher titer, as high as 1:150 in normal serum, and the titer is frequently increased in infections with other species of dysentery bacilli. Flexner agglutinin has, then, very little diagnostic value unless it is to high titer and agglutinins for Shiga and typhoid bacilli are absent. Agglutinins to the Newcastle bacillus, *Bact. ambiguum* and *Bact. alkalescens* occur only in low titer. *Bact. sonnei* is often agglutinated to titers as high as 1:50 by normal serum, and, though the titer may occasionally be very high in infection, sometimes little or no agglutinin response is apparent.

Despite the immunological response evidenced by the appearance of agglutinins, there is little or no resistance to second attacks and no method

of active immunization, other than to the soluble toxin of *Bact. shigae*, has yet proved effective in bacillary dysentery. In this connection it may be recalled that the infection is confined to the lumen of the intestine as a rule and in a very real sense remains outside the body. Bacillary dysentery, then, differs from typhoid fever in the important respect that the bacilli do not invade the tissues and consequently are not exposed to the action of circulating antibody and phagocytic cells. Possibly this accounts, at least in part, for consistent failure to develop an effective immunity to infection with these bacilli.

Pathogenicity for Lower Animals.—As pointed out above, the cell substance of the dysentery bacillus is markedly toxic to experimental animals upon parenteral inoculation. The Shiga bacillus is by far the most toxic. Considerable quantities of the other dysentery bacilli are required to elicit symptoms of toxemia. Feeding with dysentery bacilli is generally not successful, though the administration of large doses of living bacilli has resulted in dysenteric symptoms in cats and *rhesus* monkeys. Lower animals apparently do not suffer from bacillary dysentery under natural conditions, although it is not uncommon to isolate *Bact. flexneri* from monkeys in the laboratory that develop enteric symptoms.

CHAPTER 22

THE CHOLERA VIBRIO AND RELATED FORMS

Family: *Pseudomonadaceae*. Tribe: *Spirilleae*. Genus: *Vibrio*. Short, bent rods, rigid, single or united end-to-end in spirals. Motile by a single (or rarely, 2 or 3) polar flagellum. Many species liquefy gelatin. Aerobic, facultative anaerobic. Non-spore-forming, gram-negative. Water forms; a few are parasites. Type species: *Vibrio comma*.

Although Asiatic cholera has doubtless smoldered endemically in parts of India for many centuries, the year 1817 marked its first considerable extension beyond the borders of that country. Europe was first invaded in 1831, and since that date a series of great epidemics has carried the disease over a large part of the civilized world. The disease was brought to New York by Irish immigrants during the pandemic of 1832–33 and in the pandemic of 1846–62 invaded the United States via New Orleans (1848) and spread up the Mississippi Valley. The fourth great pandemic, that of 1864–75, affected Asia, Africa, Europe and America. The causal agent of the disease, the cholera vibrio, was discovered in 1883 by Koch[1] in the intestinal discharges of cholera patients. Similar microorganisms were described by later workers in infected water and elsewhere, and now a number of species are known. In general, however, these other vibrios are non-pathogenic and have been studied in terms of their relation to the cholera vibrio and are, therefore, not particularly well known. Of what is now a fairly sizable group, only two species are pathogenic, the microorganism discovered by Koch and variously termed *Spirillum cholerae asiaticae* (Koch), *Spirillum cholerae*, *Vibrio cholerae*, the comma bacillus, and *Vibrio comma* (Bergey); and a vibrio pathogenic for pigeons and guinea pigs, *Vibrio metchnikovii*.

VIBRIO CHOLERAE

Morphology and Staining.—The cholera vibrio is a short, slightly curved and twisted rod, 1.5 to 3 μ in length and 0.4 to 0.6 μ in breadth. It may occur singly or in chains which have the appearance of short spirals or S-shaped forms (two cells). The straight and spiral threads formed in the pellicle of liquid gelatin cultures are usually regarded as involution forms. Cultures that have been maintained for a long time on agar often lose the curved form and appear as straight rods, but resume the more characteristic form when passed through animals. The vibrios are actively motile by a single polar flagellum which is shorter than the flagella of most bacteria. Spores are not formed. The cholera vibrio stains readily with the ordinary aniline dyes and is gram-negative.

Colonies on agar media are similar to those of the other enteric bacilli, but may be distinguished from those of *Bact. coli* by their thin, opalescent

[1] Koch: Ber. klin. Wchnschr., 1884, *21*:477, Brit. Med. Jour., 1884, *ii*:403, 453.

appearance. They are 1 to 2 mm. in diameter, low, convex and grayish-yellow in color, with a finely granular consistency which is accentuated under low magnification. Some strains are hemolytic on blood agar while others are not (see below).

Physiology.—The cholera vibrio is strongly aerobic and only very sparse growth appears under anaerobic conditions, and then on prolonged incubation. It grows over the temperature range of 16° to 42° C. with an opti-

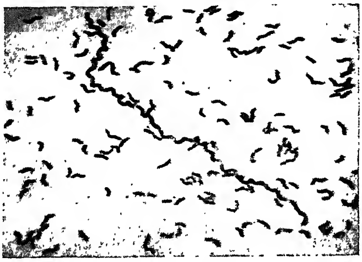


Fig. 78.—*Vibrio cholerae;* broth culture two days old; fuchsin stain; × 1000 (Fränkel and Pfeiffer).

mum growth at 37° C. An alkaline reaction is essential for good growth; the bacteria will grow over a *p*H range of 6.4 to 9.6 and are usually cultivated at an alkaline reaction, *p*H 7.8 to 8.0. This marked tolerance for alkali is taken advantage of in the preparation of selective media for the isolation of the cholera vibrio, the *p*H of such media usually being about 9.5. They are not nutritionally fastidious and may be grown in peptone water.

THE HEIBERG FERMENTATION TYPES OF VIBRIOS

Sugar	Type					
	I	II	III	IV	V	VI
Sucrose	+	+	+	+	−	−
Mannose	+	−	+	−	+	−
Arabinose	−	−	+	+	−	−

Fermentation reactions are variable and a number of carbohydrates, including dextrose, levulose, galactose, maltose, sucrose and mannitol, may be fermented with the production of acid but no gas. Lactose, inulin and dulcitol are not attacked. Heiberg[2] has studied the fermentation reactions of the vibrios at some length and has arrived at six fermentative types on the basis of the fermentation of sucrose, arabinose and mannose which are known as the Heiberg types. Type I is characterized by the fermentation of

[2] Heiberg: C. R. Soc. Biol., 1934, *115:*984.

sucrose and mannose but not arabinose, and contains all of the cholera vibrios and some non-cholera varieties. Starch is hydrolyzed. Both coagulated serum and gelatin are liquefied. Stab cultures in gelatin often develop a small turnip-shaped area of liquefaction at the surface, which by evaporation of the fluid leaves a bubble-like depression, while in some growth little or no liquefaction occurs along the needle puncture. Other vibrios besides the cholera vibrio, however, produce this same type of liquefaction. Growth in milk does not produce any visible change for some time, but a slow peptonization without coagulation appears on continued incubation. Hydrogen sulfide and indol are produced and nitrates are reduced to nitrites. The addition of sulfuric acid to a culture of the cholera vibrio in nitrate-peptone broth results in the development of a red color—the so-called "cholera-red reaction." This is, of course, the nitroso-indol reaction and is

Fig. 79.—*Vibrio cholerae* (left) and the vibrio of Finkel and Prior (right), showing the single polar flagella. × 2000 (Kral).

given by any bacterium, as the colon bacillus for example, that both reduces nitrate and produces indol. Other vibrios give this reaction also.

The question of the hemolytic activity of the cholera vibrio has been of considerable importance in relation to its differentiation from the closely related El Tor vibrios. The term hemolysis, when applied to the vibrios, refers to hemolytic activity as determined by the *Grieg test* in which a 3 per cent suspension of goat erythrocytes is mixed with an equal quantity of twenty-four-hour broth culture of the vibrio and read after two and four hours' incubation at 37° C. By this test the cholera vibrio is non-hemolytic.[3] As indicated above, however, some strains of the cholera vibrio are hemolytic on blood agar. The apparent contradiction has been resolved by van Loghem[4] in particular, who has shown that in the case of this bacterium at least, blood plate hemolysis is a hemodigestive process and basically different from the liberation of hemoglobin from suspensions of erythrocytes.

[3] For a recent study see Read, Pandit and Das: Indian Jour. Med. Res., 1942, *30*:183.
[4] *Cf.* van Loghem: Centralbl. f. Bakt., I Abt., Orig., 1913, *67*:410.

The resistance of the cholera vibrio to various injurious influences is not great. It is killed by moderately high temperatures (ten minutes at 55° C.) and is destroyed quickly by chemical disinfectants. It is particularly sensitive to drying; if a drop of broth culture be dried on a slide, the vibrios are all dead in about two hours. It does not survive long in association with the ordinary saprophytic bacilli of soil and water, and whether it is able to multiply outside the body in impure water is uncertain. Upon the surface of vegetables and fruits kept in a cool, moist place the vibrios may remain viable for from four to seven days. The slight resistance of the cholera vibrio, especially its sensitiveness to drying, explains the rapid and complete disappearance of cholera in once infected localities, and also the circumstance that the disease is rarely, if ever, air-borne.

The biochemical structure of the vibrios has been studied at some length by Linton and his co-workers.[5] They have described three types of poly-

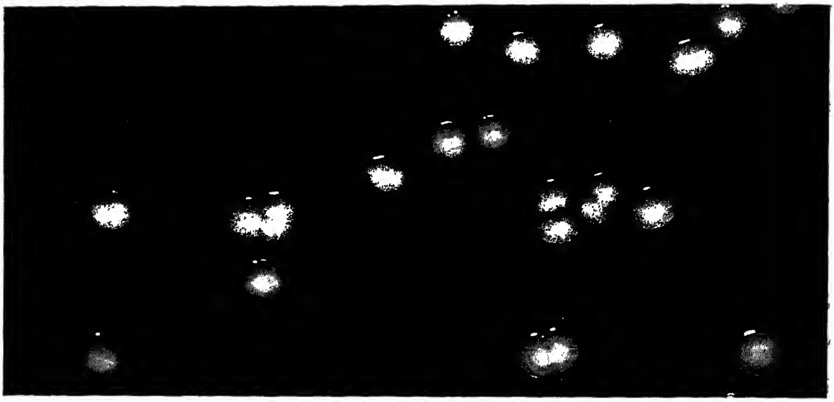


Fig. 80.—Colonies of the cholera vibrio on Vedder and Van Dam's medium. The magnification and lighting accentuate the granular structure of the colonies which does not usually appear so pronounced. × 6.

saccharide and two types of protein present in the cholera and related vibrios. The polysaccharides were differentiated on the basis of their hydrolysis products; that designated type I was made up of galactose and an aldobionic acid (galactose and glucuronic acid), type II of arabinose and aldobionic acid, and type III was a polymer of glucose. The two protein types, both globulins, were differentiated on the basis of racemization, *i. e.*, optical activity of the hydrolyzed protein before and after alkali racemization. These substances occurred in combinations to give six groups of vibrios, the cholera vibrios falling into groups I and II which contained the same protein but polysaccharide types I and II respectively. The significance of these observations is by no means clear. It is evident, however, that the polysaccharide bears no relation to the immunological character of the vibrios for the cholera vibrios are immunologically homogeneous

[5] Summarized in the review by Linton: Bact. Rev., 1940, *4*:261.

(see below), and it is inconceivable that galactose- and arabinose-containing polysaccharides should show identical immunological specificity. Apparently neither the protein nor polysaccharide fractions are associated with the endotoxin.

Toxin.—The evidences of profound toxemia in human infections with the cholera vibrio are strongly suggestive of the formation of toxic substances by this microorganism. It was early shown by Pfeiffer[6] that, while filtrates of young broth cultures were only slightly toxic, vibrios killed with heat or chloroform from young agar cultures were markedly toxic to guinea pigs on intraperitoneal inoculation. The toxicity of cell-free filtrates of old broth cultures led a number of the earlier investigators to the conclusion that a soluble exotoxin is produced. It is now established, however, that no exotoxin is formed and the toxicity of old broth cultures is attributable to dissolution of the vibrios and the liberation of endotoxin.

The endotoxin is heat stable and it was early found by Metchnikoff to be diffusible through collodion sacs.[7] The isolation of a toxic protein has been reported by some workers[8] and the toxicity has been extracted by the trichloracetic acid method.[9] On the basis of the latter observations it has been assumed that the toxin is a polysaccharide-lipid complex similar to those isolated from typhoid and dysentery bacilli; this conclusion is not, however, supported by unequivocal evidence. More recently it has been found[10] that the cholera toxin is associated, and possibly identical, with a dialyzable phospholipid which may be extracted from the intact vibrios with organic solvents such as alcohol, ether and chloroform.

Though in general the pharmacological activity of the bacterial endotoxins is not characteristic, it has been reported[11] that the intravenous inoculation of experimental animals with toxic preparations produces a diarrhea and pathologic changes in the intestine and kidneys not unlike those observed in human cholera. It has been shown by Burrows, Wagner and Mather[12] that purified toxin markedly increases the permeability of the isolated strip of intestine to fluids. In any case, the cholera toxin seems to have a special affinity for the epithelium and causes a shredding of the epithelium of the intestine and gallbladder in human cholera.

The antigenicity of the cholera toxin has been open to some question for, in general, the antitoxic qualities of cholera antiserum are only feeble

[6] Pfeiffer: Ztschr. f. Hyg., 1892, *11*:393; Pfeiffer and Wassermann: *ibid.*, 1893, *14*:46.

[7] For more recent studies see Basu, Chaudhury and Basu: Calcutta Med. Jour., 1940, *36*:571; and Banerjee: Jour. Indian Med. Assn., 1942, *11*:95.

[8] Galeotti: Centralbl. f. Bakt., I, Orig., 1912, *67*:225; Sanarelli: Ann. Inst. Pasteur., 1920, *34*:370; Hahn and Hirsch: Centralbl. f. Bakt., I, Orig., 1927, *104*:211; *ibid.*, Klin. Woch., 1927, *6*:312; *ibid.*, Ztschr. f. Hyg. u. Infektionskr., 1929, *110*:355.

[9] Checcacci: Boll. Inst. Sieroterap. Milanese, 1939, *18*:391; Raynal, Lieou and Feissole: Rev. Immunol., 1939, *5*:317; *ibid.*, 1940, *6*:132; Damboviceanu and Barber: C. R. Soc. Biol., 1940, *133*:501; Gallut: Ann. Inst. Pasteur, 1943, *69*:123.

[10] Burrows: Proc. Soc. Exp. Biol. Med., 1944, *57*:306.

[11] Hahn and Hirsch: Klin. Woch., 1928, *7*:2483; Gosh: C. R. Soc. Biol., 1933, *112*:1176; Pham: *ibid.*, 1935, *119*:78.

[12] Burrows, Wagner and Mather: Proc. Soc. Exp. Biol. Med., 1944, *57*:311.

at best. Some workers have, however, reported[13] the preparation of effective antitoxin. The purified lipid toxin has been found to be antigenic in that antisera to it show marked protective qualities as measured by the mouse protection test.[14]

Antigenic Structure.—The first demonstration of bacterial agglutination with homologous immune serum by Gruber and Durham[15] was the agglutination of the cholera vibrio and the typhoid bacillus. It was soon apparent that the agglutination reaction was specific and it has been used for the identification of the cholera vibrio since. The antigenic structure of the cholera and related vibrios has, therefore, been of considerable interest in connection with the differentiation of *V. cholerae.*

The existence of serologic types within this group was shown by Japanese workers. Kabeshima[16] demonstrated the existence of two types by cross absorption and designated one "original" or J, and the other "variant" or F. A third serologic type was found by Nobechi[17] which he designated "intermediate" or "middle" since it appeared to have affinities with the other two. The "original" and "variant" types, sometimes called "end types" are now known as Inaba and Ogawa types respectively, and the "middle" type is called Hikojima. All three are equally capable of causing epidemic cholera, but Inaba is the most common, practically the only type in India, and Hikojima is found least often.

It was shown by Balteanu[18] that the vibrios, like other motile bacteria, contain both heat-stable, somatic, or *O* antigen and heat-labile flagellar or *H* antigen. Shousha[19] found that the *O* antigen of the cholera vibrio is specific, while the *H* antigen is shared with a variety of non-cholera vibrios such as water vibrios and the like. Gardner and Venkatraman[20] have carried this analysis further and found that most vibrios can be differentiated into six groups which they designated by Roman numerals based on the specificity of the *O* antigen. Group I contains all of the cholera vibrios and some El Tor vibrios. Serological identification of the cholera vibrio, therefore, requires the use of specific *O* antiserum. Since the El Tor vibrios are hemolytic, the cholera vibrio is defined as a non-hemolytic member of Group I. Gardner and Venkatraman account for the specific agglutination of earlier workers on the basis that *O* agglutination occurs more rapidly than *H* agglutination and hence was observed first. The differentiation of the Japanese types is a consequence of slight differences in the *O* antigens. A curious terminology has crept into the cholera literature with respect to agglutination; it is said that a strain which is agglutinated by specific antiserum is "agglutinable" while those vibrios which are not agglutinated by cholera

[13] Pottevin: Bull. Soc. Path. Exot., 1913, *6:*409; Horowitz: Ztschr. f. Immunitätsf., 1913, *19:*44; Bail: *ibid.*, 1916, *25:*248; *ibid.*, 1917, *26:*97; Hahn and Hirsch: Centralbl. f. Bakt., I, Orig., 1927, *104:*211; *ibid.*, Ztschr. f. Hyg. u. Infektionskr., 1929, *110:*355; Andu and van Niekerk: Centralbl. f. Bakt., I, Orig., 1929, *112:*519.

[14] Burrows, Mather, Wagner and McGann: Proc. Soc. Exp. Biol. Med., 1944, *57:*308

[15] Gruber and Durham: Muench. Med. Woch., 1896, *43:*285.

[16] Kabeshima: as early as 1912; original articles in Japanese, cited by Nobechi.

[17] Nobechi: Sci. Rept. Govt. Inst. Inf. Dis., Tokyo, 1923, *2:*29, 43.

[18] Balteanu: Jour. Path. Bact., 1926, *29:*251.

[19] Shousha: Jour. Egypt. Med. Assn., 1931, p. 438.

[20] Gardner and Venkatraman: Jour. Hyg., 1935, *35:*262.

antiserum are called "inagglutinable" despite the fact that they are readily agglutinated by homologous antiserum.

Variation.—The cholera vibrio is well known for its tendency to develop bizarre involution forms. These are found not only in old cultures and cultures grown under somewhat adverse conditions, such as increased salt concentrations, but also are produced by the inclusion of substances such as glycine or alanine in the medium.[21] Such changes in morphology are also associated with the usual type of S-R dissociation which occurs readily under the influence of bacteriophage, lithium chloride, etc., the rough variants showing distinctive colonial character, spontaneous agglutination in salt solution, etc.

The immunological changes associated with the S-R dissociation have been studied in some detail by White. He[22] has found that four groups of rough variants are immunologically distinguishable, and his Group A appears to correspond to the Group I of Gardner and Venkatraman. Further degeneration to the so-called ρ variants results in a loss of specific *O* antigen. An independent type of variation, rugose-non-rugose, may be induced, and the rugose strains of S, R and ρ variants contain an *O* antigen that is common to both members of Group A and vibrios of other groups.[23] A somatic protein antigen has been isolated by White[24] which is common to all known variants and shows wide cross-precipitin reactions throughout the vibrio group, although it appears not to be concerned in the agglutination reaction.

Pathogenicity for Man.—The causal connection between Asiatic cholera and the microorganism discovered by Koch has been demonstrated by a number of laboratory accidents. One of the first occurred in Koch's laboratory, and other infections resulting from the accidental swallowing of cultures of the cholera vibrio have been noted since. In one instance the swallowing was deliberate; Pettenkofer and Emmerich voluntarily swallowed a small quantity of broth culture of "Koch's vibrio" and as a result developed cholera.

Both laboratory cases of cholera and those cases contracted naturally in the course of epidemics are marked by great differences in the susceptibility of different individuals. This is probably a consequence in part of innate individual differences in natural resistance, and in part to predisposing factors. Fatigue, the excessive use of alcohol, and various factors leading to mild, non-specific gastro-intestinal derangements, predispose in a marked degree to attacks of cholera.

The incubation period is short; it is usually given as three to five days but may occasionally be as short as twenty-four hours. When the vibrios have entered the small intestine and established themselves they multiply rapidly and, with autolysis and dissolution of the cells, endotoxin is liberated and symptoms appear. There are two clinical stages, the first that of *vomiting* and *profuse diarrhea* with the characteristic ricewater stools con-

[21] Gordon and Gordon: Jour. Path. Bact., 1943, *55*:63.
[22] White: Jour. Hyg., 1935, *35*:347.
[23] White: Jour. Path. Bact., 1940, *50*:160.
[24] *Ibid.*, 1940, *50*:165.

taining flakes of mucus, shed epithelial cells and enormous numbers of the vibrios. With the tremendous loss of fluid dehydration and hypochloremia become marked, and collapse, the *algid stage*, ensues with circulatory failure, subnormal temperature and anuria. Death may occur in either the first or second stage. The case fatality rate is over 50 per cent in untreated cases and about 30 per cent in treated cases.

At autopsy the small intestine shows marked destruction of the epithelial lining and a characteristic subepithelial edema. It is generally said that ulceration does not occur in cholera, but Goodpasture[25] has shown that desquamation of the epithelium is followed by ulceration if the patient survives long enough. Other changes are not characteristic and include a prominence of lymphoid tissue and cloudy swelling of the kidneys.[26]

Cholera in man appears to be largely, if not entirely, a toxemia, for the infection is confined to the lumen of the intestine and vibrios are found in the organs only rarely. It is not altogether clear whether toxin is absorbed. In the opinion of many the profound dehydration and hypochloremia and consequent impairment of renal function are sufficient to cause the clinical symptoms and pathology.

Carriers.—On recovery the vibrios disappear rapidly and are discharged for only a short time. In the series of 200 cases studied by Ying[27] 98 per cent were negative by the end of the second week, only a few giving positive cultures as late as the third and fourth weeks. There appears to be no chronic carrier state established in cholera. The statements in the literature regarding chronic carriers refer to persons who discharge "agglutinable" vibrios of the El Tor type; a chronic carrier of typical virulent cholera vibrios has never been observed.[28] It is probable that casual carriers occur during epidemics but in no instance has this been demonstrated bacteriologically. While the convalescent carrier, and possibly the casual carrier, may play some part in the spread of the disease, the case, especially in the incubation period, is probably by far the most important source of infection.

Bacteriological Diagnosis of Asiatic Cholera.—As indicated above, the vibrios are present in the ricewater stools in very large numbers and can usually be isolated from fresh specimens without difficulty. At times they are present in sufficient numbers that they can be found in stained smears, preferably made from a flake of mucus. Both enrichment and direct streaking of agar media are used for culture. For enrichment a few drops of the stool are added to a tube of alkaline (*p*H 8.0 to 9.0) peptone water. The vibrios grow much more rapidly than the other intestinal bacteria and after six to eight hours' incubation form a thin film of growth on the surface of the medium which can be smeared and stained, and streaked on agar.

A number of agar media have been used for isolation of the cholera vibrio, including starch-phenolphthalein agar and the alkaline blood medium

[25] Goodpasture: Philippine Jour. Sci., 1923, *22*:413.

[26] The pathology is summarized briefly by Banerjee: Jour. Indian Med. Assn., 1939, *8*:39.

[27] Ying: Chinese Med. Jour., 1940, *58*:595.

[28] The carrier question is discussed by Couvy: Bull. Office Internat. d'Hyg. Publique, 1933, *25*:1149.

of Dieudonné. The latter has been the most generally used. In its preparation an excess of alkali is added and the poured plate must be ripened, *i.e.*, left partly open in the incubator for twenty-four hours to allow the evolved ammonia to escape. With storage the *p*H continues to drop and the medium becomes less selective and unsatisfactory. The alkaline hemoglobin medium of Vedder and Van Dam[30] is buffered with glycine, requires no ripening, and the *p*H remains constant at 9.5 with storage. It possesses all the advantages and none of the disadvantages of Dieudonné's medium. It is sometimes implied that these media with a *p*H at the upper limit of tolerance of the cholera vibrio are so highly selective that few if any other intestinal bacteria will grow on them. This is not true, for micrococci may be found in abundance on such plates; the writer has observed counts of 1000 million bacteria per gram of normal guinea pig feces on both Dieudonné's and Vedder and Van Dam's media. However, colonies of the cholera vibrio are readily distinguished and may be picked and identified by slide agglutination with monospecific *O* antiserum. With clinically typical cholera in an epidemic this is sufficient identification. A negative Grieg test, fermentation of sucrose and mannose and failure to ferment arabinose, and positive indol and nitrite (cholera-red) reactions further substantiate the identification.

Epidemiology.—As indicated earlier, the great endemic focus of Asiatic cholera is in India, especially in the delta of the Ganges River. Recent evidence has indicated that there is also an endemic area in China, in the valley of the Yuan River which flows into the Yangtse River through Tun Ting Lake.[31] Whether this is recently established or recently discovered is not known. In any case, the disease spreads out in epidemic form from these foci each year, occurring regularly throughout India, in the Yangtse Valley and along the China coast. The entire Far East is affected at one time or another, especially China and French Indo-China, and the disease appears from time to time in Manchuria and Japan. The last general outbreak in the Philippine Islands was in 1934; a few cases appeared in 1935 and 1936. The geographic distribution differs greatly from year to year and that indicated in Fig. 81 is only an approximation.

Infection spreads to the west especially via the pilgrimages; pilgrims going from the delta of the Ganges to Arabia and Mecca transmit it to pilgrims from Egypt and Algiers. The quarantine stations at El Tor and Basra (see map) serve to keep the disease out of Europe in normal times though it seeps through into Iran and Iraq with some frequency. There was cholera in Europe during the Balkan War and the First World War and the disease occurred in epidemic form in Eastern Europe in 1921 and 1922. There has been no cholera in the United States proper since 1911.

As in the other enteric infections, the connecting link in the dissemination of cholera is between infected feces and the mouths of susceptible persons. In consequence, the disease is frequently water-borne and may be transmitted by any food ordinarily consumed in the raw state. The quanti-

[30] Vedder and Van Dam: Nederl. Tijdschr. v. Hyg. Microbiol. en Serol., 1932, *7*:197.
[31] Robertson and Pollitzer: Trans. Roy. Soc. Trop. Med. Hyg., 1939, *33*:213.

tative importance of contact infection is not known. Cholera differs from the other enteric diseases, however, in the highly explosive character of the outbreaks, which is attributable to the short incubation period, the high case fatality rate, and its rapid and permanent disappearance when the outbreak has subsided. The last is due in part, perhaps, to the inability of the vibrio to survive long apart from the host, but more important is the limited duration of the convalescent carrier state and the absence of chronic carriers. In a sense cholera is one of the easiest to control of the highly contagious diseases for it cannot spread when sanitary facilities, *i.e.*, sewage disposal, water supply, etc., are in efficient operation. A striking instance

Fig. 81.—The approximate distribution of Asiatic cholera. The precise distribution cannot be shown because of great variability from time to time. This represents an approximate distribution during the decade 1930–1940. Based in part on map prepared by Army Medical Intelligence, 1943, epidemiological reports of the League of Nations, and various authors. (Based on Goode *Base Map* No. 205. By permission of the University of Chicago Press.)

of this was reported in the Balkan War in 1913 in which infection was widespread in the Bulgarian Army about Sofia, but cases in the capital were largely imported and the disease showed little tendency to spread there; Sofia was efficiently sewered and had an excellent water supply.[32] Contrariwise, the famous Broad Street Pump epidemic in London in 1854 was made possible by the common use of a continuously infected well,[33] and the Hamburg epidemic of 1892 arose from infection of the river Elbe. The Hamburg water supply was raw river water, but the neighboring city

[32] Eckert: Berlin. Klin. Woch., 1913, *50*:2326.
[33] *Snow on Cholera*. The Commonwealth Fund. New York. 1936.

of Altona purified the water by sand filtration and remained free of the disease.[34]

Immunity.—Antibodies, agglutinins and bacteriolysins, are produced by infection with the cholera vibrio but have no diagnostic utility because of late and irregular appearance. It is usually said that recovery from an attack of cholera confers an immunity to subsequent infection, but neither the quality of this immunity nor its duration is definitely known. It seems probable that the immunity is not of a high order and that it does not persist more than a year or two at the most.

Active immunization of man with vaccines has been carried out for many years. The first vaccine was that of Haffkine (1893) who used a living attenuated culture in Spain and India.[35] A heat-killed vaccine was used by Kolle, and was reported to give good results in Japan. Sensitized vaccines, *i.e.*, vibrios treated with antiserum, have been investigated also.[36] The cholera vaccine used at the present time in this country and in India is a saline suspension of killed vibrios, half the Inaba variety and half the Ogawa variety. Cholera vaccines are usually heavier than the vaccines prepared from other enteric bacilli, and contain 8 to 10 billion vibrios per cubic centimeter. Usually two doses of 1 cc. each are given a week apart, and a booster dose of 1 cc. every six months.

It is not clear how effective active immunization is. Much of the reported evidence is open to criticism and not convincing. The difficulties of carrying out an adequately controlled experiment in man are, of course, very great indeed. The earlier work was examined by Greenwood and Yule[37] who found it unconvincing on the whole, but there was a slight presumption in favor of inoculation. Most subsequent reports have been favorable with a few definitely unfavorable. In general it would appear that immunization confers some immunity to infection, but probably not of a high order. It is generally agreed that cholera is as severe in the inoculated as in the uninoculated and the therapeutic use of antisera is apparently without effect. It may be noted that the problem of active immunization to cholera is very similar to that of bacillary dysentery (p. 421).

Pathogenicity for Lower Animals.—Under natural conditions domestic animals and the animals used in the laboratory never contract cholera. It is possible to produce enteric infection with the cholera vibrio in the guinea pig by oral administration (by stomach tube) of very large numbers of the vibrios either suspended in alkaline buffer or following oral administration of bicarbonate, provided that intestinal motility has been reduced by prior administration of tincture of opium. The suckling rabbit may be similarly infected and is considerably more susceptible than the guinea pig, but after weaning cannot be infected. It is not clear how closely related these experimental infections are to human cholera.

[34] Koch: Ztschr. f. Hyg., 1893, *14*:393.

[35] A history of this work is given by Haffkine: *Protective Inoculation Against Cholera.* W. Thacker & Co. London. 1913.

[36] *Cf.* Takenouchi: Jour. Inf. Dis., 1920, *26*:441.

[37] Greenwood and Yule: Proc. Roy. Soc. Med., Sec. Epidemiol. and State Med., 1915, *8*:113.

The guinea pig is susceptible to intraperitoneal inoculation of living vibrios and "intraperitoneal cholera" has been extensively studied by the earlier workers. Recently Griffitts[38] has shown that the virulence of the cholera vibrio for mice by intraperitoneal inoculation may be markedly enhanced by suspension in 5 per cent mucin; as few as 50 living vibrios may kill by this method. This will undoubtedly be of considerable utility in assay of virulence and active and passive immunity, but would appear to be quite unrelated to the enteric infection of man.

Active or passive immunization of experimental animals to parenteral inoculation of the cholera vibrio is highly successful, and the usual immunization procedures yield high titer antisera. The immunity so conferred is apparently almost wholly an antibacterial immunity for little antitoxic activity may be detected. It was shown by Pfeiffer[39] that lysis of the vibrios by immune serum occurs readily. The *Pfeiffer phenomenon* may be demon-

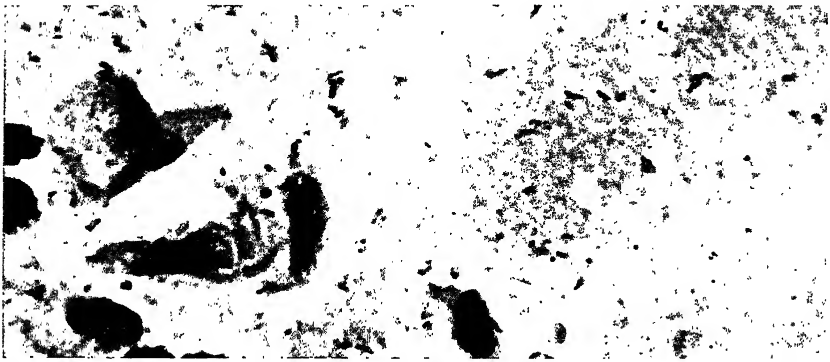


Fig. 82.—*Vibrio cholerae* in peritoneal exudate of a guinea pig. Note the swollen and aberrant forms. Gram stain; × 1250.

strated in either actively or passively immunized guinea pigs by intraperitoneal inoculation of living vibrios; if peritoneal fluid is withdrawn from time to time and examined, it will be found that the vibrios lose their motility, become swollen and misshapen and then disintegrate. The phenomenon may be produced *in vitro* with potent antiserum and complement.

PARACHOLERA VIBRIOS AND NON-CHOLERA VIBRIOS

For the most part as an adjunct to the study of the cholera vibrio, a considerable number of vibrios have been isolated from water and from the feces of individuals suffering from mild diarrheal disease, so-called cholera nostra. Some of these have been given place names such as *Vibrio danubicus*, *Vibrio ghinda*, *Vibrio massauah*, etc. A phosphorescent vibrio, *Vibrio phosphorescens*, has been isolated from water, and *Vibrio proteus* from human feces. All of these differ immunologically from the cholera vibrio, *i.e.*,

[38] Griffitts: Pub. Health Repts., 1942, *57*:707.
[39] Pfeiffer: Ztschr. f. Hyg., 1894, *18*:1; *ibid.*, 1895, *20*:198.

belong to *O* Groups other than I, and have faded from general interest. Some may possibly have feeble pathogenic properties but in tropical and subtropical regions where diarrheal disease is common (and of varied etiology) and great numbers of vibrios are present in water used for drinking, it is not surprising that they are occasionally found in fecal specimens though unrelated to a disease condition. In general, the group of vibrios is very poorly known from the biological point of view.

The El Tor Vibrios.—The best known of the so-called paracholera vibrios are those which were first isolated at the Tor quarantine station in 1906[40] from pilgrims suffering from diarrheal disease. A considerable number of strains was isolated at Tor in 1930 and 1931 by Doorenbos, and apparently these vibrios can be isolated with some frequency there.[41] Very many, though not all, of the El Tor strains are "agglutinable," that is to say, agglutinate with anticholera sera, and have been shown to belong to *O* Group I by Gardner and Venkatraman.[20] The agglutinable strains differ from the cholera vibrio only in that they are actively hemolytic as noted earlier; a number of physiological tests, such as the Voges-Proskauer reaction, have been said to differentiate the two but none has proved to be reliable.

The relationship of these vibrios to Asiatic cholera has been a matter of considerable interest; aside from general considerations, their presence raises a specific practical question at quarantine stations. Doorenbos[42] is strongly of the opinion that two types of cholera may be distinguished, the epidemic form caused by the classic cholera vibrio, and the endemic form caused by atypical variants of *V. cholerae* such as the El Tor vibrio; inherent in this is the assumption that the El Tor vibrios may become typical *V. cholerae*. This view is not generally accepted and it is believed that the El Tor vibrio, sometimes given the specific name *Vibrio El Tor*, has some pathogenic powers and may be causally associated with diarrheal disease, but neither the vibrio nor the disease are related to *V. cholerae* and Asiatic cholera in the sense of Doorenbos.

The Celebes Vibrio.—The question of the pathogenicity of the hemolytic agglutinable vibrios was raised again by the occurrence of an epidemic, apparently of cholera, in Celebes in 1937 and 1938 which was reported by de Moor.[43] About 400 strains of the vibrio were isolated and all proved to be hemolytic and agglutinable with cholera antiserum; van Loghem[44] found representative strains to be indistinguishable from the El Tor vibrio. With the outbreak of war in Europe and the Far East these strains were no longer available and do not seem to have been studied further. This epidemic, in which many cases were clinically indistinguishable from cholera though some were mild, is convincing evidence of a high degree of pathogenicity of at least some strains of the hemolytic agglutinable vibrios.

[40] Gotschlich: Ztschr. f. Hyg., 1906, *53*:281.

[41] For example, see Marras: Ann. d'Igiene, 1940, *50*:1.

[42] Doorenbos: Rev. d'Hyg. et de Med. Preventive, 1936, *58*:595, 675, 736; *ibid.*, 1937, *59*:22, 105; Jour. Egypt. Med. Assn., 1938, *21*:279.

[43] de Moor: Bull. Office Internat. d'Hyg. Publique, 1938, *30*:1510; see also de Vogel: *ibid.*, 1940, *32*:556.

[44] van Loghem: Bull. Office Internat. d'Hyg. Publique, 1938, *30*:1520.

Other Pathogenic Vibrios.—A number of other vibrios are known which produce disease in animals but which are apparently not pathogenic for man. *Vibrio metchnikovii* was isolated[45] from fowls suffering from an epidemic disease resembling fowl cholera. It closely resembles the cholera vibrio morphologically and physiologically and is highly pathogenic for guinea pigs and pigeons while the cholera vibrio is not pathogenic for the latter. It differs from *V. cholerae* immunologically and neither is agglutinated nor lysed by anticholera serum. An infectious abortion of sheep caused by *Vibrio fetus* was described by McFadyean and Stockman[46] in England and also occurs in this country but is not prevalent. The vibrio is relatively difficult to cultivate and is biochemically inactive, fermenting none of the sugars. It is not pathogenic for guinea pigs. An epidemic disease of carp and other fish caused by a vibrio designated *Vibrio piscium* has been described by David.[47] The vibrio resembled the cholera vibrio morphologically, and was immunologically related to it. A vibrio to which no formal name was assigned has been described by Doyle[48] as associated with swine dysentery. This vibrio was very difficult to cultivate and its relationship to the other vibrios is not as yet clear.

[45] Gamaléia: Ann. Inst. Pasteur, 1888, *2*:482.
[46] McFadyean and Stockman: Report of the Departmental Committee appointed by the Department of Agriculture and Fisheries to inquire into epizootic abortion. 1909. Part 1, p. 15.
[47] David: Centralbl. f. Bakt., I Abt., Orig., 1927, *102*:46.
[48] Doyle: Amer. Jour. Vet. Res., 1944, *5*:3.

CHAPTER 23

BRUCELLA[1]

(Undulant Fever, Contagious Abortion of Cattle)

Family: *Parvobacteriaceae*. Tribe: *Brucelleae*. Genus: *Brucella*. Small, gram-negative short bacilli or coccobacilli. Usually non-motile. Require enriched media. Carbohydrates not fermented. Gelatin not liquefied. An alkaline reaction is produced in milk and other culture media. Brownish growth on potato. Strict parasites producing various infections in man and the higher animals. Type species: *Brucella melitensis*.

In 1887 Bruce,[2] while investigating the human disease known as Malta fever, Mediterranean fever or undulant fever, discovered a microorganism in the spleen of fatal cases of the disease which he designated *Micrococcus melitensis*. A disease of goats transmissible to man, this affection not only is common on the island of Malta, where British garrisons have been often seriously affected, but occurs also on neighboring islands and on the shores of the Mediterranean Sea, and has been occasionally reported from India, South Africa, the Philippines and the West Indies. It was first brought to attention in the United States about 1911.

In 1897 Bang,[3] in Denmark, isolated a microorganism responsible for a contagious abortion in cattle, an affection now commonly known as Bang's disease, which he termed *Bacillus abortus*. The isolation and cultivation of this bacterium in the United States were first recorded by MacNeal and Kerr.[4]

These two diseases, one primarily of goats and secondarily of man, and the other, one of cattle, were long studied quite independently, and apparently no connection between the two was recognized prior to the work of Evans[5] in 1918. This worker demonstrated the remarkably close morphological, cultural and serological relationship existing between these bacteria which are now recognized as being intimately related to one another.

In 1914 Traum[6] isolated from fetuses prematurely expelled from sows a bacterium which is now known to be closely related to the bacterium of Bang's disease and that of undulant fever. Regarded as three species, these bacteria have been given the generic name of *Brucella* and are designated as *Brucella melitensis*, *Brucella abortus* and *Brucella suis*. Infection with these bacteria is often termed "brucellosis."

Morphology and Staining.—The *Brucella* are small coccoid or short bacillary forms varying from 0.4 to 3.0 μ in length and from 0.4 to 0.8 μ in

[1] For a detailed discussion of these bacteria see Huddleson: *Brucellosis in Man and Animals*. The Commonwealth Fund. New York. 2nd ed., 1939.

[2] Bruce: Practitioner, 1887, *39*:161.

[3] Bang: Ztschr. f. Tiermedizin, 1897, *1*:241.

[4] MacNeal and Kerr: Jour. Inf. Dis., 1910, *7*:469.

[5] Evans: Jour. Inf. Dis., 1918, *22*:580.

[6] Traum: Ann. Rept. Chief, Bur. Animal Ind., U. S. Dept. Agr., 1914, p. 30.

breadth. Some variability is noted, and both coccoid and bacillary forms may appear intermingled. There is a greater tendency to the coccobacillary form in *Br. melitensis* than in *Br. suis*, with *Br. abortus* intermediate between the two, but no distinction can be made on a morphological basis. The microorganisms usually occur singly or in pairs, and in cultures short chains may be found. The smooth forms are encapsulated[7] but spores are not formed, and these bacteria are non-motile.

On semisolid media the colonies are small, circular, convex, amorphous, smooth, glistening and translucent. No pigment is formed, but the growth of *Br. melitensis* becomes brown in older cultures and the browning extends down into the medium. This browning is shown by some strains of *Br. abortus* also.

Brucella may be stained by the usual aniline dyes, but there is a tendency

Fig. 83.—*Brucella melitensis*, pure culture. Note the coccobacillary appearance. Fuchsin; × 1050.

toward irregular staining and, in some cases, bipolar staining. They are gram-negative.

Physiology.—The nutritive requirements of these bacteria are relatively complex and best growth is obtained on enriched media such as liver infusion broth or agar. Kerby and Calder[8] have reported that a milk-tryptose-crystal violet medium is superior to liver infusion broth or tryptose broth in primary culture. *Brucella* have been cultivated on amino acid synthetic media; some strains require nicotinamide, thiamin and pantothenic acid while others require biotin also.[9] McCullough and Dick[10] have obtained growth in glucose-inorganic salt media containing these bacterial vitamins. No growth occurs at 6° C. or 45° C. and the optimum temperature is 37° C. Neither acid nor gas is produced from carbohydrate media, although it may be shown that glucose is utilized to a small extent and its inclusion generally favors growth. Nitrates are reduced, and growth in

[7] Huddleson: Jour. Amer. Vet. Med. Assn., 1940, *96*:1708; Mickle: Jour. Inf. Dis., 1940, *66*:271.

milk is accompanied only by a slowly increasing alkalinity. Gelatin is not liquefied and indol is not formed. The optimum *p*H is 6.6 to 6.8.

Hydrogen Sulfide.—All three species produce hydrogen sulfide but differ in that *Br. suis* produces it in abundance, *Br. abortus* to a lesser extent and *Br. melitensis* to only a slight degree. It may be noted that ammonia is produced to a greater extent by *Br. melitensis* than by the other two species.

Carbon Dioxide.—These bacteria are aerobic, and *Br. melitensis* and *Br. suis* may be grown on primary isolation under the usual aerobic conditions. *Br. abortus*, however, requires incubation in an atmosphere containing 25 per cent carbon dioxide on primary isolation. Subsequent transfers from the primary growth must be incubated in 10 per cent carbon dioxide, but after a number of transfers *Br. abortus* adapts itself to ordinary aerobic growth.

Dyes.—The *Brucella* species differ from one another in their suscepti-

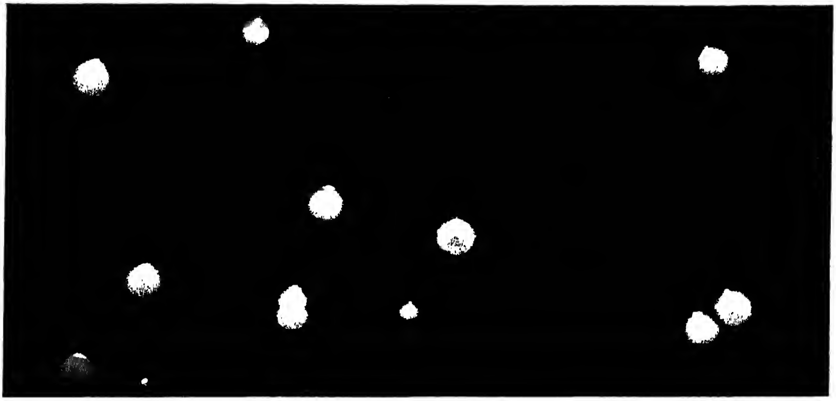


Fig. 84.—*Brucella melitensis.* Pure culture on liver infusion agar. × 4.

bility to the bacteriostatic effect of dyes, and their ability to grow on liver infusion agar containing thionin or basic fuchsin is a characteristic of considerable practical value in their differentiation. *Br. melitensis* and *Br. abortus* will grow in the presence of basic fuchsin in a dilution of 1:25,000, but *Br. suis* is completely inhibited. *Br. melitensis* and *Br. suis* will grow in the presence of thionin in a dilution of 1:50,000, while *Br. abortus* will not. These and other cultural differences are summarized in the accompanying table.

The *Brucella* show the usual susceptibility to heat and disinfectants. A point of some practical importance is the rapid death of these bacteria at pasteurizing temperatures; both *Br. abortus* and *Br. suis* are killed in three minutes at 143° to 145° F. They persist in soil, water and dust for one to

[8] Kerby and Calder: Jour. Bact., 1940, *40*:637.

[9] Koser and Wright: Jour. Inf. Dis., 1942, *71*:86; McCullough and Dick: *ibid.*, 1942, *71*:193, 198.

[10] McCullough and Dick: Proc. Soc. Exp. Biol. Med., 1943, *52*:310.

two months but disappear within ten days in milk, presumably in part as a consequence of the presence of acids formed by other bacteria. In this connection it is of interest that these bacteria are able to survive two hours or more in milk mixed with gastric juice.

CULTURAL DIFFERENCES OF BRUCELLA SPECIES

	CO_2 Required for Growth	H_2S Production	Growth in Presence Of	
			Basic Fuchsin 1:25,000	Thionin 1:50,000
Br. melitensis	−	±	+	+
Br. abortus	+	+	+	−
Br. suis	−	++	−	+

Antigenic Structure.—Each of the three Brucella species contains two antigens, designated as *A* and *M*. *Br. melitensis* contains a relatively large amount of *M* and a small amount of *A*, while both *Br. abortus* and *Br. suis* contain large amounts of *A* and small amounts of *M*.[11] The ratio of *A* to *M* is said to be about 20:1 in the case of *Br. abortus* and 1:20 in the case of *Br. melitensis*.[12] It is possible, then, to differentiate *Br. melitensis* from *Br. abortus* and *Br. suis* by serological methods, *i.e.*, agglutination, but *Br. abortus* and *Br. suis* cannot be differentiated from one another in this way. In practice monospecific sera, *i.e.*, sera adsorbed to remove the small amount of antibody common to the other immunological type, should be used. These antigens are both heat-stable, and their chemical nature is as yet unknown.[13]

Variation.—The *Brucella* species dissociate relatively easily to give rise to the rough form. The S-R transformation is accompanied by a change to a rough colonial type, a loss of virulence and alteration in immunological specificity. The last begins to take place before morphological changes are apparent and has been a source of considerable difficulty in the serological typing of these bacteria; the altered immunological types have been termed *paramelitensis* or *para-abortus* strains. It is, therefore, essential to use only smooth cultures in the serological differentiation of these bacteria.

A number of tests have been devised for the detection of antigenic variants. The rough forms are, of course, spontaneously agglutinated in saline. Slightly rough strains may be detected by their agglutination upon boiling in saline for two hours (thermo-agglutination test) or by incubating saline suspensions of the bacteria with basic fuchsin or trypaflavine at 37° C. for two hours; the rough form agglutinates in the presence of the dye while the smooth form remains evenly suspended. According to Huddleson,[14]

[11] Wilson and Miles: Brit. Jour. Exp. Path., 1932, *13*:1.
[12] Miles: Brit. Jour. Exp. Path., 1939, *20*:63.
[13] *Cf.* the investigations of Miles and Pirie: Brit. Jour. Exp Path., 1939, *20*:83, 109, 278; Biochem. Jour., 1939, *33*:1709, 1716.
[14] *Cf.* Munger and Huddleson: Jour. Bact., 1938, *35*:255.

however, none of these gives consistent results, and he has proposed an opsonocytophagic test in which bacterial suspension is mixed with citrated normal guinea-pig or human blood, incubated for thirty minutes, smeared and stained. Smooth strains show only a slight degree of phagocytosis while the rough bacteria are phagocytosed to a considerable extent.

Pathogenicity for Lower Animals.—Brucellosis is primarily a disease of domestic animals and is only secondarily communicated to man; the chief animal reservoirs are goats, cattle and swine. It is of some interest that host specialization of the parasites has taken place, giving rise to the three species or, as some prefer to regard them, varieties of *Brucella*.

Goats.—Goats may be artificially infected with *Br. melitensis* by almost any route, and it is probable that under natural conditions the vaginal discharge at the time of aborting and shortly thereafter plays an important part in the dissemination of the infection. Agglutinins appear in the blood of artificially inoculated pregnant goats by the third or fourth day, and the titer rises rapidly to perhaps 1:1000 within forty-eight hours and reaches a peak of 1:2000 or thereabouts by the twelfth day of infection. Just before the peak in agglutinin titer, a bacteremia is initiated which persists for perhaps one month. This acute generalized infection becomes localized during the second month after the termination of the pregnancy during which the animal was infected. In most cases the bacteria do not persist in the udder and uterus after the fifth month following termination of pregnancy. A second pregnancy does not, as a rule, cause an exacerbation of the disease, but in some cases the infection may remain localized in the area of the genital tract for several years.

The most obvious clinical symptom of infection is abortion, although this need not occur. Pyrexia is apparent within forty-eight hours of the generalized infection, and there is a slight diarrhea. The placenta is not retained, but a copious vaginal discharge is frequently observed for two or three weeks after kidding. In lactating goats the milk may be physically altered and appear in extreme cases as a clear fluid containing suspended clots.

Immature goats are highly resistant to the infection, and kids born of infected dams may not be infected and commonly do not become so in spite of the ingestion of enormous numbers of *Brucella* in the milk. Nonpregnant mature goats are also resistant to infection and respond to artificial inoculation with only a low and transient agglutinin titer in the blood serum.

Brucellosis in sheep is similar to that in goats.

Cattle.—Brucellosis in cattle is most commonly an infection with *Br. abortus* although both *Br. melitensis* and *Br. suis* have also been found. The microorganism may gain entrance by a variety of routes, including direct inoculation into the vagina, by way of the conjunctiva, through the unbroken skin or via the alimentary tract. The primary symptom of the disease is abortion of the fetus by pregnant cattle.[15] The time elapsing between initial infection and abortion varies from three weeks to four months,

[15] Abortion may, of course, result from other infections. See the discussion by Gilman: Cornell Vet., 1939, *29*:153.

and the period of gestation at which abortion may take place varies from two to nine months. Cattle do not abort, however, unless infected during pregnancy and even then not all abort—perhaps 30 per cent—or the cattle may become sterile. Subsequent pregnancies may proceed normally in spite of persistence of the infection; second abortions are not common, and third abortions are rare.

The bacilli may be found in the blood in perhaps 10 per cent of the cases and are very likely consistently present during the acute infection. Early in the infection the bacteria are found in the lymph glands about the head and intestines, by the end of the first month they are found all through the body, and by the end of the third month have localized in the mammary glands and are found only in the udder. The invasion of the udder results in an acute or chronic inflammation with lesions in the alveoli and interalveolar connective tissue and, when the lymph glands are involved, a chronic lymphadenitis. Chronic infection of the udder may persist indefinitely without significant differences in the quality of the milk[16] and bacilli may be excreted over a long period of time, perhaps for life. The uterus, on the other hand, frees itself of the bacteria relatively soon and the vaginal discharges do not contain the bacilli for an extended period.

Animals infected during pregnancy show an agglutinin titer ranging from 1:200 to 1:1000 which falls slowly over a period of six months or so. Cattle that continue to excrete bacilli in the milk generally show persistent agglutinin titers of 1:200 or more although a titer of 1:50 has diagnostic significance. Agglutinins are also present in the milk and may be demonstrated in the whey after clotting with rennin. Infected animals become sensitized to the bacillary substance, and a skin reaction may be elicited by the intradermal injection of a preparation of *Brucella* protein designated as *abortin* or *brucellergen*. As in the case of the young goats, calves are relatively resistant to the infection.

Swine.—*Brucellosis* of swine seems to be always due to infection with *Br. suis*, though these animals may be artificially infected with *Br. abortus*. In contrast with cattle, the males are commonly infected and abortion in infected females is less frequent than in cows; about 50 per cent of swine abortions are due to unknown causes, not brucellosis.[17] The clinical symptoms may be mild or lacking, and in a number of instances there has been no outward evidence of the disease in an infected herd. Under natural conditions infection probably takes place via the alimentary tract. The bacilli are eliminated with aborted fetuses and vaginal discharge, urine, semen and milk.

Other Animals.—A number of other animals have been found to be naturally infected with *Brucella*. There is some evidence that the disease of horses known as fistula of the withers or poll evil is a *Brucella* infection; both *Br. abortus* and *Br. suis* have been isolated from cases of the disease. Stone[18] found that 9.5 per cent of horses tested in New York City gave positive serological reactions. *Brucella* infections of fowl have been re-

[16] *Cf.* Emminger and Schlam: Amer. Jour. Vet. Res., 1943, *4*:100.
[17] McNutt and Leith: Michigan State Coll. Vet., 1943, *4*:28.
[18] Stone: Jour. Amer. Vet. Med. Assn., 1941, *99*:118.

ported; in one instance *Br. suis* was isolated from several naturally infected birds, but the disease is probably not common. Dogs may also be naturally infected; *Br. suis* and *Br. abortus* have been isolated. Wild rats may be infected artificially, and *Br. abortus* has been isolated from a naturally infected rat. Natural infection of rabbits with *Br. melitensis* has also been reported.[19] Of the usual laboratory animals guinea pigs are readily infected and are most often used for experimental purposes. Rabbits, mice and other animals may also be infected. A disease resembling undulant fever in man has been produced in *rhesus* monkeys.

Pathogenicity for Man.—Man is susceptible to infection with these three species of *Brucella*, but infections with *Br. melitensis* and *Br. suis* are usually more severe than those with *Br. abortus*. The incubation period of undulant fever in man is highly variable and relatively long; according to Hardy, Frant and Kroll,[20] it may range from one week to not less than four months. The case fatality is low—2 to 3 per cent. It may have varied clinical manifestations and 5 types are recognized: (1) the intermittent type with shifting articular rheumatism, weakness, night sweats and a temperature near normal in the morning but rising to 101° to 104° F. in the evening, in which the patient remains in bed in the latter part of the day; (2) the ambulatory type with much the same symptoms but to a mild degree; (3) the undulant type, generally *melitensis* infections, characterized by step-like increases in the temperature from day to day to a maximum and, after a time, gradual decrease in temperature and possibly successive repetitions of this sequence of events; (4) the malignant type, almost always *melitensis* infections, in which the temperature is high and sustained with an extreme hyperpyrexia before death; and (5) an atypical chronic type which may take the form of muscular stiffness, gastric disturbances, and various neurological symptoms. In general, undulant fever is a disease of relatively long duration, one to four months, and relapses during convalescence are not infrequent. In a chronic form undulant fever may be difficult to diagnose.[21]

Brucellosis in man is a generalized infection as a rule, while in lower animals it is a localized infection, particularly in cattle. The bacilli may be isolated from the blood stream in man and the development of agglutinins is a diagnostic aid. Man also becomes sensitized to the cell substance of the bacteria, a hypersensitivity that is sometimes manifested as skin eruptions which may be macular or resemble the rose spots of typhoid fever. Localization may occur, however, and meningitis and meningo-encephalitis are probably not so rare as has been supposed, while in some instances orchitis, cholecystitis, endocarditis and other local manifestations have been reported. Pulmonary lesions with infiltration of the hilar glands or lung tissue proper are occasionally observed.[22] *Brucella* infection may be as-

[19] For a discussion of brucellosis in wildlife see the review by Katz: Jour. Amer. Vet. Med. Assn., 1941, *99*:24.

[20] Hardy, Frant and Kroll: Pub. Health Repts., 1938, *53*:796.

[21] *Cf.* Evans: Amer. Jour. Trop. Med., 1939, *19*:319; Pub. Health Repts., 1938, *53*:1507.

[22] Althoff and Rating: Klin. Woch., 1943, *22*:597.

sociated with abortion and mastitis in the human female in rare instances.[23]

Epidemiology.—Brucellosis in man is probably always acquired from infected domestic animals; man-to-man transmission is a possibility but rarely, if ever, occurs. The commonest modes of infection in the United States are, first, the use of raw milk from infected cattle and, second, direct contact with the flesh of infected animals, both cattle and swine. As indicated above, animals may be readily infected via the alimentary tract, and it is not unreasonable to suppose that man is infected in this way also The discharge of *Br. abortus* in the milk of infected cattle, then, provides the opportunity for infection when the milk is ingested in the raw state and in many instances undulant fever is acquired in this way. *Br. abortus* has been found in certified milk in a number of localities.[24] The pasteurization of milk, of course, provides adequate protection from this source of infection.

The penetration of the unbroken skin by *Brucella* has been pointed out earlier. Man may be infected by the handling of the tissues of diseased animals or by close contact with other infectious material; presumably the bacilli enter through minute abrasions in the skin, or possibly through the intact skin. Employees of slaughterhouses, veterinarians, sausage-makers and butchers are, of course, particularly exposed to infection by this means and, in fact, the incidence of brucellosis in this group is disproportionately high. It is probable that most infections with *Br. suis* are acquired in this manner, although in some instances cattle are infected with this species and man acquires a *suis* infection via raw cow's milk.

Laboratory infections with *Brucella* are very common and even the most skilled workers have acquired undulant fever through working with these bacteria. Meyer and Eddie[25] have reviewed 74 cases up to 1940 of which 44 occurred in competent bacteriologists. These infections are, in all probability, a consequence of handling infectious material and penetration of the skin by the microorganisms.

Undulant fever may also be acquired by drinking raw goat's milk, but infections with *Br. melitensis* from these animals are thought to be relatively infrequent in the United States. Evans,[26] however, has found a number of cases in North Carolina, Kansas and Texas, and it is known to occur in the Southwest in general where goat's milk is consumed.

It has been shown experimentally that brucellosis may be transmitted by mosquitoes and biting flies, but at present there is no indication that this mode of transmission is of any significance in nature. Water is apparently not a vehicle of transmission; the single water-borne outbreak that has been reported[27] was in the nature of a laboratory accident.

The prevalence of human brucellosis is not known with any degree of

[23] The clinical aspects of human brucellosis are discussed in detail by Harris: *Brucellosis.* Paul B. Hoeber. New York. 1941.

[24] See, for example, Hasley: Jour. Inf. Dis., 1930, *46*:430.

[25] Meyer and Eddie: Jour. Inf. Dis., 1941, *68*:24.

[26] Evans: Pub. Health Repts., 1937, *52*:295.

[27] Huddleson and Munger: Amer. Jour. Pub. Health, 1940, *30*:944.

precision. As a consequence of better diagnosis, the number of reported cases has steadily increased from 24 in 1925 to 2497 in 1937. During the period 1930–1941 29,594 cases of brucellosis were reported in the United States, an average annual rate of 1.87 per 100,000 population.[28] The disease is found in rural areas for the most part and the incidence in urban areas is very low. The incidence is high in the midwestern states and, in geographical distribution, tends to parallel the extent of the hog-raising industry. The incidence of brucellosis in cattle is relatively high (10 per cent?) and more or less uniform throughout the country. Swine brucellosis is not so common; about 3 per cent of hogs show significant agglutinin titers.

Bacteriological Diagnosis of Brucellosis.[29]—The laboratory diagnosis of brucellosis involves the demonstration of the causative microorganism and of specific antibodies. When the bacterium can be isolated and identified the diagnosis is solidly established, but the presence of antibodies indicates only an immune response to what may have been a past rather than present infection and is, therefore, only suggestive.

Brucella is most often found in the blood stream in man, particularly in the pyrexial period, but cannot always be isolated. Blood is added to tryptose broth in 2 to 5 cc. amounts and incubated in an atmosphere containing 10 per cent CO_2. The enrichment culture should be subcultured at four-day intervals and, if subcultures are negative, carried for a period of not less than three weeks. For subculture agar plates of liver infusion or tryptose agar should be inoculated. The bacteria may be identified by agglutination with antiserum; differentiation between *Br. melitensis* and *Br. abortus-Br. suis* may be made by agglutination with type-specific antiserum, and the culture tested for H_2S production and growth in the presence of thionin and basic fuchsin.

Agglutinins may be titrated in the usual way but the interpretation of the test may be difficult unless the titer is very high. Probably a titer below 1:80 is not significant, while titers as high or higher than 1:1000 are seldom if ever observed in the absence of infection. Even a very high titer is not conclusive evidence of infection, of course. Conversely, some infected individuals show only low agglutinin titers, perhaps 1:20. Huddleson feels that the opsonophagocytic test for the presence of opsonins is a much more reliable measure of the immune response. Citrated whole blood is mixed with bacterial suspension, incubated without agitation for thirty minutes, and a film of the mixture spread on a glass slide. The film is dried rapidly and stained with Hastings' stain (Wright's stain may be used) and examined for the presence of the bacilli in the neutrophils. The test is negative when no phagocytosis occurs, slight when 1 to 20 bacilli are found per cell (25 counted), moderate when the phagocytosed bacilli are 21 to 40 per cell, and marked when there are more than 40 per cell. In the last case, the neutrophils are usually packed with bacilli and there are too many to be counted. Marked phagocytosis in 40 per cent or less of the cells examined indicates infection, and in more than 60 per cent, an immune reaction.

[28] Jordan, Borts, Harris and Jennings: Amer. Jour. Pub. Health, 1943, *33*:773.

[29] See the general discussion by Foshay: Amer. Jour. Clin. Path., 1940, *10*:176; and by Harris: Bull. New York Acad. Med., 1943, *19*:63.

Immunity.[30]—The resistance of calves and non-pregnant cows to clinically apparent brucellosis is clearly an expression of natural immunity, though the older animals respond to the microorganism with the production of antibodies and the development of an increased resistance to subsequent infection. Man likewise appears to have a high degree of natural resistance to the infection and it is probable that there are many more infections than clinical cases of brucellosis. In the series studied by Huddleson and Munger[27] in which exposure to infection was known, only about half the individuals showing evidence of infection by an immune response had clinically apparent disease.

In man the immune response is evidenced by the appearance of agglutinins, opsonins and hypersensitivity to preparations (*brucellergen*) of the cell substance of the bacteria. It is not clear, however, that this response is associated with an increased resistance, *i.e.*, effective immunity, to the infection. Prophylactic inoculation with *Brucella* vaccines is not practical in man, though the available evidence indicates that it is effective in cattle. The therapeutic use of antisera or vaccines in human brucellosis has given disappointing results.

BRUCELLA BRONCHISEPTICA

This microorganism is very similar to *Br. abortus*, but is *motile* and highly aerobic. It does not produce hydrogen sulfide. It is immunologically related to *Br. melitensis* and *Br. abortus*, but can be separated from them by agglutinin-absorption tests. It also resembles *Bacillus pertussis* both culturally and immunologically. Originally isolated from dogs ill with distemper, it is not now generally believed to stand in any causal relation to that disease.[31] It is, however, frequently found as the cause of bronchopneumonia in guinea pigs and other rodents.

[30] See the review by Huddleson: Bact. Rev., 1942, *6*:111.
[31] See, for example, Hardenbergh: Jour. Amer. Vet. Med. Assn., 1925, *68*:309.

CHAPTER 24

PASTEURELLA

(Hemorrhagic Septicemia; Plague; Tularemia)

Family: *Parvobacteriaceae.* Tribe: *Pasteurelleae.* Genus: *Pasteurella.* Small gram-negative bacilli, often ovoid; bipolar staining. Carbohydrates attacked slightly or not at all. Gelatin not liquefied. Parasites of man and certain higher animals and birds. Type species: *Pasteurella avicida.*

The term "hemorrhagic septicemia" was applied by Hueppe[1] to a group of highly fatal infectious diseases of the lower animals in which large and small hemorrhagic areas are found in the subcutaneous tissues, serous membranes, muscles and lymph glands, and throughout the internal organs. The causative bacteria constitute a group of closely related, biochemically inactive, non-motile, gram-negative forms showing bipolar staining. The first of these bacteria to be studied was the etiological agent of fowl cholera which Pasteur used in his early studies on immunity. Others have been described as producing hemorrhagic septicemias in various lower animals. Regarded by some as but a single species under the name *Pasteurella pluriseptica*, these bacteria are usually separated into species which differ from one another in host adaptation and minor fermentation reactions. They have been given names derived from the kind of animal in which they were found.

The species of bacteria causing the hemorrhagic septicemias of lower animals are:

Pasteurella aviseptica (*Bacillus avisepticus, Bacterium avisepticum, Pasteurella avicida**)—the fowl cholera bacillus. Pathogenic for birds and mammals.

Pasteurella muriseptica (*Bacillus murisepticus, Bacterium murisepticum, Pasteurella muricida**)—found in naturally infected wild rats and pathogenic for rabbits, guinea pigs, mice and rats but not for chickens or pigeons.

Pasteurella lepiseptica (*Bacillus lepisepticus, Bacterium lepisepticum, Pasteurella cuniculicida**)—the bacillus occurring in contagious nasal catarrh or "snuffles" of rabbits as a clinical or latent infection. Produces septicemia in rabbits upon parenteral inoculation. Pathogenic for chickens and mammals.

Pasteurella suiseptica (*Bacillus suisepticus, Bacterium suisepticum, Pasteurella suilla**)—the bacillus of swine plague. Pathogenic for mice, rabbits and birds.

Pasteurella boviseptica (*Bacillus bovisepticus, Bacterium bovisepticum, Pasteurella bollingeri**)—produces a hemorrhagic septicemia in cattle, hogs and horses and is found in deer and wild hogs.

[1] Hueppe: Ber. klin. Wchnschr., 1886, *23*:753, 776, 794.

* The starred names are those of Bergey's 1939 classification.

So far as is known, the diseases produced by these bacteria are not ordinarily communicable to man. Rare cases of human infection with bacilli of this group have, however, been reported.[2] Closely related to this group and classified as species of *Pasteurella* are the bacilli of plague or "black death" and of tularemia or "rabbit fever." *Past. pestis* differs culturally from the hemorrhagic septicemia bacilli in that it grows in the presence of

Fig. 85.—*Pasteurella suiseptica*. Smear from a pure culture. Fuchsin; × 1050.

bile, does not ferment sorbitol, and does not produce indol or hydrogen sulfide. Unlike the other *Pasteurella* species, *Past. tularensis* requires enriched media for growth.

PASTEURELLA PESTIS—THE PLAGUE BACILLUS

Plague prevailed extensively throughout Europe during the Middle Ages. It has been estimated that 25,000,000 persons, or one-quarter of all inhabitants of Europe, perished in the "great mortality" or "black death" of the fourteenth century (1348–49). Few diseases have left so deep a mark on general literature. Boccaccio's *Decameron* contains one of the most vivid descriptions of the plague ever written, and Defoe's fictitious[3] *Journal of the Plague Year* provides a realistic picture of the devastation of London in 1665 by an outbreak of "black death" in which 70,000 persons perished.

For reasons that may be only partly conjectured the plague has had irregular periods of quiescence and recrudescence. Western Europe has been practically free from the plague since the middle of the eighteenth century, and the disease began its first great extension in modern times with its appearance in 1893 in Hongkong and in 1896 in Bombay. During recent years the plague has caused terrible loss of life in British India; official statistics show that in the period from 1896 to 1918 more than 10,000,000 deaths were due to this disease. In October, 1899, a case was

[2] The hemorrhagic septicemia bacilli are discussed at length by Regamey: *Les infections humaines a B. bipolaris septicus (Pasteurelloses)*. Huber. Berne. 1939.

[3] Defoe was only four years old in the year of the great plague.

recorded at Santos, Brazil; this is thought to be the first occurrence of the plague in the Western Hemisphere. Plague first appeared in the United States in San Francisco in 1900; it is assumed that it was introduced by infected rats from the Orient. The infection apparently spread to ground squirrels and other wild rodents in the western part of the country. From 1900 to 1939 there were 499 reported cases of plague with 314 deaths in 8 states.[4]

The plague bacillus, *Pasteurella pestis*, was discovered almost simultaneously by Yersin[5] and by Kitasato.[6]

Morphology and Staining.—The plague bacillus is a short, plump, ovoid rod 0.3 to 1.25 μ in length. In the body fluids the bacilli may occur in pairs, but long chains are rare and, in general, there is no characteristic arrangement. The bacilli are non-motile and are encapsulated.[7] Involution forms are common, especially in older cultures, and coccus shapes, large

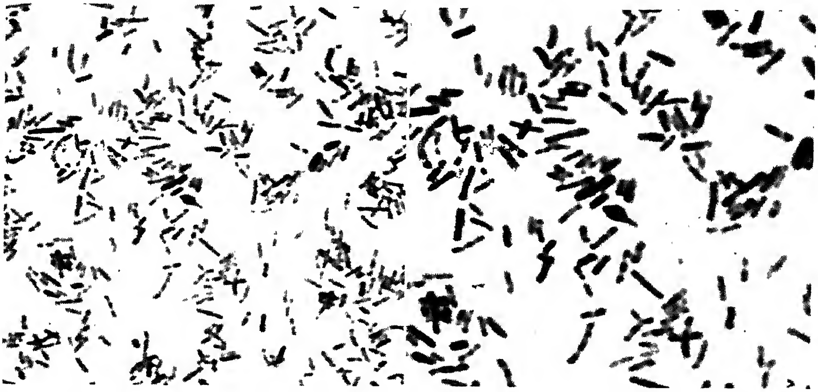


Fig. 86.—The plague bacillus. Smear from pure culture; fixed in methyl alcohol and stained with methylene blue to show bipolar staining. Note the involution forms present even at twenty-four hours' incubation. Left, × 1050; right, × 1800.

rods and gigantic swollen forms may be observed. The tendency of the plague bacillus to aberrant morphology is accentuated by cultivation on media containing 3 to 4 per cent sodium chloride; the appearance of involution forms in twenty-four-hour cultures on salt-containing media has been regarded by some as a characteristic of differential value.

Colonies on nutrient agar or gelatin have a delicate, drop-like appearance, with a round, granular center and a thin, granular, uneven margin.

The plague bacillus is uniformly gram-negative and shows a marked tendency toward polar staining, *i.e.*, there are heavily stained areas at the ends of the cell separated by a lightly stained area in the center (see Fig. 86). For good bipolar staining the smear should be air-dried and fixed

[4] Hampton: Pub. Health Repts., 1940, *55*:1143.
[5] Yersin: Ann. Inst. Pasteur, 1894, *8*:662.
[6] Kitasato: Lancet, 1894, *ii*:428.
[7] See the study by Sokhey: Jour. Path. Bact., 1940, *51*:97.

in alcohol. The usual aniline dyes, such as methylene blue, are satisfactory. The plague bacillus is best demonstrated in tissue sections by a polychrome stain.

Physiology.—*Past. pestis* is not nutritionally fastidious, and growth occurs on all the ordinary culture media, although in peptone water it is very poor. Some strains will grow in amino acid media without bacterial vitamins while others require nicotinamide or thiamin or both.[8] Unlike most of the bacteria pathogenic for man, a temperature of 25° to 30° C. is more favorable than one of 37° C., and the limiting temperatures for growth are −2° C. and 45° C.[9] In any case, the colonies on semisolid media grow slowly and never attain a large size. The plague bacillus is aerobic and facultatively anaerobic.

Sugar fermentations are variable, and a small amount of acid but no gas is produced. Neither coagulated serum nor gelatin is liquefied, and indol is not produced. Nitrates are reduced to nitrites, and a small amount of hydrogen sulfide is formed. On potato and in milk multiplication is slow and scanty; milk is rendered slightly acid but is not curdled.

One of the most characteristic cultural features is observed in the growth in broth. When the surface of this medium is covered with a layer of oil and flasks are left undisturbed for five or six days after inoculation, long, delicate filaments are formed which hang down from the surface into the depths of the clear broth, like the stalactites that depend from the roof of a grotto. Not all cultures of *Past. pestis* show stalactite growth in equal degree, and, on the other hand, a similar formation has been observed in cultures of other bacteria; the stalactite formation, therefore, while highly characteristic, especially when broth is seeded directly from fresh plague buboes, is not specific.

The plague bacillus does not exhibit any marked resistance to deleterious influences. Exposure to drying, particularly at the higher summer temperatures, kills it within a short time. The bacillus is quite sensitive to the action of sunlight and chemical disinfectants; it is killed, for example, by 0.5 per cent phenol in ten to fifteen minutes and by heating to 55° C. in about the same time. Cultures kept in the refrigerator, however, remain viable over long periods of time. In general, the life of *Past. pestis* outside the animal body is precarious, and the bacillus seems to disappear speedily from soil, water and buried cadavers.

Toxins.—No soluble toxic substances are formed by the plague bacillus, though the toxicity of old broth cultures to experimental animals on parenteral inoculation is indicative of the toxicity of the bacillary cell substance.

Pathogenicity for Man.—Plague in man appears most commonly in two forms, the bubonic or glandular plague and plague pneumonia. In the bubonic type the symptom-complex is characteristic, and diagnosis on clinical grounds is relatively simple. From the buboes, which may be either primary or secondary, bacilli may pass over into the blood; in fatal cases the bacteria often multiply in the blood extensively. The case fatality is

[8] Berkman: Jour. Inf. Dis., 1943, *71*:201; Doudoroff: Proc. Soc. Exp. Biol. Med., 1943, *53*:73.

[9] Sokhey and Habbu: Jour. Bact., 1943, *46*:25.

60 to 90 per cent. A primary plague septicemia can also probably occur. There are sometimes subcutaneous hemorrhages. During the plague epidemics in the Middle Ages such hemorrhages seem to have been more frequent than at present, and the dark spots to which they give rise were the origin of the popular name of "black death."

Plague pneumonia occurs secondary to the glandular infection and may be transmitted to give rise to primary plague pneumonia. Pneumonic plague is usually fatal. In this variety the sputum may contain enormous numbers of plague bacilli, and the infection is spread from man to man by droplets. Because of this direct spread pneumonic plague is by far the more dangerous type. The extensive outbreak of pneumonic plague in Manchuria in 1910–12 is said to have caused approximately 60,000 deaths. the case fatality being practically 100 per cent.

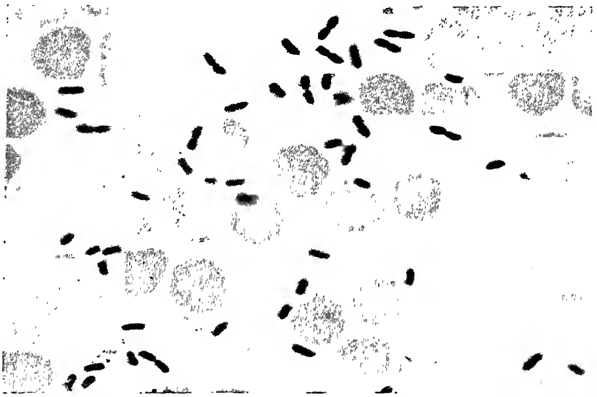


Fig. 87.—The plague bacillus in the blood of an infected mouse. × 2000 (Douglas and Wheeler).

A primary infection of the skin with the plague bacillus sometimes occurs (cutaneous plague) but does not seem to be common. Cases of mild plague, the so-called "pestis minor," are met with in some epidemics, but it is probable that healthy persons do not carry plague bacilli for long, if at all. The occurrence of intestinal plague in man has never been clearly established.

Epidemiology.—Plague is primarily a disease of rats and other rodents and is spread from rat to rat and from rat to man through the bite of infected fleas. At least three species of rats, *Epimys norvegicus*, the common gray sewer rat; *E. rattus*, the black house rat and ship rat; and *E. alexandrinus*, the Egyptian rat, are known to be capable of receiving infection. The Indian rat flea, *Xenopsylla cheopis*, is probably the most important rat flea in the dissemination of plague, but *Ceratophyllus fasciatus*, the common rat flea of North America and Europe, may also transmit the disease.

The black rat lives in close association with man, and its presence in

dwelling houses makes it a particularly dangerous vector of plague. It is commonly believed that the great prevalence of the plague in Europe in the Middle Ages was caused by the advent of the black rat brought in from Asia Minor in the ships of the Crusaders. The black rat in turn was largely driven out of Europe by the arrival of the fiercer gray rat from the north. This second change in the character of the rat population is thought to explain in large part the practical disappearance of the plague from Europe. Probably the advances made over the medieval type of dwellings with their "rush-strewn floors" were also a factor.

Fig. 88.—Plague bacilli in the infected flea; sections stained with methylene blue. The bacilli appear as dark stained masses. *1*, Flea on the ninth day after infection. 2, Eighteenth day after infection; note the stomach and proventriculus packed with bacilli. *3*, Twenty-second day after infection; note the large mass of bacilli and the swollen proventriculus. *4*, Twenty-third day after infection; the proventriculus is further enlarged. (Douglas and Wheeler.)

Although both the feces and the urine of infected rats sometimes contain plague bacilli, the infection is not transmitted from animal to animal under experimental conditions in the absence of fleas. The blood, however, often contains enormous numbers of the bacilli—as many as 100 million per cc. have been found—and the flea becomes infected through feeding on this infectious blood. When the plague bacilli are taken into the stomach of the flea they undergo multiplication but do not penetrate into the other parts of the body. On microscopic examination large masses of bacilli may be seen in the infected flea; the development of the infection is illustrated in Fig. 88 from Douglas and Wheeler.[10] The bacilli are discharged with the

[10] Douglas and Wheeler: Jour. Inf. Dis., 1943, *72*:18.

feces, and human infections may result from rubbing these fecal droplets into the bite as it is scratched. In some infected fleas the bacilli multiply so rapidly that they mechanically obstruct the proventriculus to such an extent that little or no food may pass. In the effort to feed, some plague bacilli are mixed with the drawn blood of the host and regurgitated into the bite, thus bringing about infection. Although rats may be infected by feeding on the carcasses of their plague-infected comrades, it is probable that, under natural conditions, transmission from rat to rat is almost always through the agency of infected fleas. It may be noted that plague and tularemia are the only bacterial diseases that are transmitted by infected insect vectors (as differentiated from mechanical transmission by

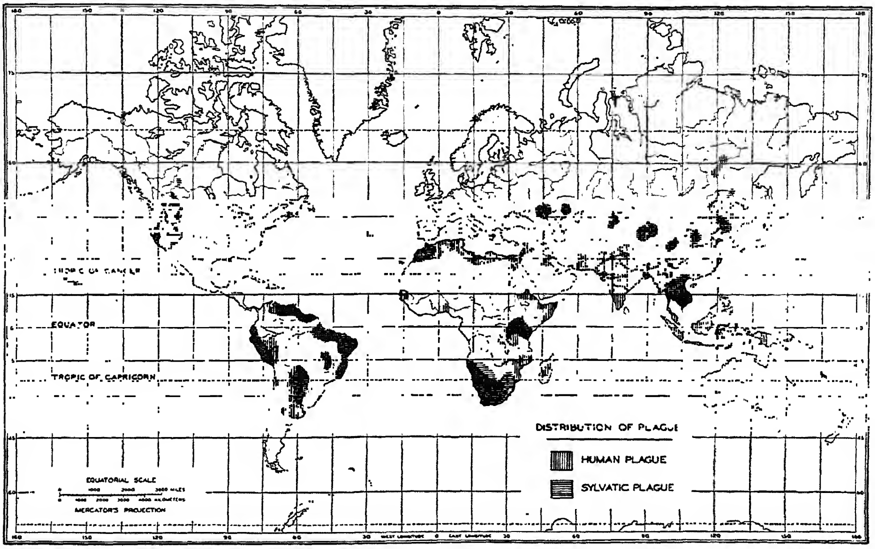


Fig. 89.—The world-wide distribution of human and sylvatic plague. Redrawn from map prepared by Army Medical Intelligence, 1943. (Based on Goode *Base Map* No. 201M. By permission of the University of Chicago Press.)

insects); the gut of most blood-sucking insects is strongly bactericidal and bacteria do not persist there more than a few hours. *Past. pestis* and *Past. tularensis*, however, are resistant to this bactericidal activity and the insect vectors may remain infective for days and perhaps weeks.

Plague is also present in other rodents. In California the native ground squirrels have proved highly susceptible to infection, and through the agency of these animals a great reservoir of infection termed *sylvatic plague* has developed.[11] The infection may be overt and occur in epizootic form, and it may also persist as an inapparent latent infection.[12] The squirrel

[11] Plague in the western part of the United States is considered in detail by Eskey and Haas: Pub. Health Bull. No. 254, 1940.

[12] Meyer *et al.*: Jour. Inf. Dis., 1943, *73*:144.

flea, *Ceratophyllus acutus*, transmits the infection to man. In other parts of the world other rodents are affected and are the means by which the disease is maintained and spread. In South Africa it is the gerbille, in Transbaikalia the tarbagan, and in Russia the spermophile that keeps the plague alive in animal reservoirs of infection. The tendency of the plague bacillus to establish these reservoirs in wild rodents constitutes a danger not fully appreciated. There is reason to believe that rodent infection is extending in several parts of the world, and there is also some experimental evidence that plague infection of man contracted from wild rodents is especially likely to assume the deadly pneumonic form.

As pointed out above, plague may assume two epidemiological forms. As pneumonic plague it is transmissible by droplet infection and its epidemiological behavior is very similar to that of the other respiratory infections. As bubonic plague it is derived from an infected rodent through the agency of the flea. In its epidemic form, bubonic plague is nearly always associated with the disease in the black rat. Infections derived from other rodents, such as the California ground squirrels, are sporadic rather than epidemic. The dissemination of bubonic plague in epidemic form, then, is determined by the closeness of the association between the infected rat population and the human population. Filth and poverty associated with a large rat population provide opportunities for transmission of the disease, and if transmission takes place on a large scale an epidemic may flare up and perhaps extend to even greater proportions as the pneumonic form develops.

Bacteriological Diagnosis of Plague.—In man the bacilli are found in material aspirated from buboes, in cultures or smears of internal organs, especially spleen, and, in pneumonic plague, in the sputum. The presence of gram-negative, bipolar staining, ovoid bacilli is highly suggestive. Blood cultures, taken late in the disease, should be cultured first in broth. Other material may be inoculated directly on blood agar and glycerol agar. Cultures may be identified by cultural and biochemical characteristics and by agglutination in plague antiserum. The bacilli show some tendency to spontaneous agglutination and the slide agglutination test is unsatisfactory. Guinea pigs may be inoculated subcutaneously or, with specimens that have undergone gross contamination and decomposition, by rubbing the material on the freshly shaven abdomen; the plague bacilli penetrate the minute abrasions while the contaminants do not. The animals die in two to five days; post-mortem findings are characteristic and include subcutaneous and general congestion, congested spleen, granular liver, and pleural effusion. The bacilli may be found in spleen smears and elsewhere and cultured. It is important that the animal be freed of ectoparasites before inoculation. Plague in rodents may be diagnosed by post-mortem findings, which are similar to those in the guinea pig, by microscopic and cultural demonstration of the bacilli, and by guinea pig inoculation.

Immunity.—Recovery from plague confers a solid immunity to subsequent infection. Experimental animals may be immunized by inoculation with suspensions of attenuated or killed plague bacilli, and numerous attempts have been made to actively immunize man in this way. One of the

first plague vaccines was that of Haffkine and consisted of heat-killed bacilli from old cultures. This and other killed vaccines have never been particularly satisfactory and do not produce an efficacious immunity in man. Inoculation with living attenuated bacilli produces a much more solid immunity in experimental animals, however, and avirulent strains appear to be as efficient immunizing antigens as virulent strains.[13] The use of living attenuated cultures for human inoculation has been attempted in the past but not continued because of possible attendant danger. More recently the use of such vaccines in man has been investigated by Grasset[14] in South Africa with encouraging results, and a similar vaccine has been used in recent years in the Netherlands Indies in more than ten million inoculations without untoward results.[15]

Antisera to the plague bacillus may be prepared by the judicious immunization of horses, or other animals, but their efficacy as therapeutic agents is doubtful.

PASTEURELLA PSEUDOTUBERCULOSIS

Past. pseudotuberculosis (*Bacillus pseudotuberculosis rodentium*) causes a disease of rodents, particularly guinea pigs. It resembles *Past. pestis* very closely but, unlike the latter, is usually actively motile at 22° C. Other differential marks are its tendency to produce alkali in milk cultures and its relatively low pathogenicity for white rats. The natural mode of infection is probably by way of the alimentary tract. Subcutaneous inoculation of guinea pigs proves fatal in two to three weeks, with caseous swellings and nodules ("pseudotubercles," which have unfortunately given this bacterium its name) in various organs. *Past. pseudotuberculosis* has been found, though rarely, in animals other than the guinea pig, and a few cases of infection have been reported in man.

PASTEURELLA TULARENSIS

Tularemia is a disease of rodents, rabbits in particular, that is transmitted to man either directly through the handling of the flesh of infected animals or indirectly through an insect vector. *Past. tularensis* (*Bacterium tularense*) was discovered by McCoy and Chapin[16] in a plague-like disease of the Claifornia ground squirrel. Tularemia in man, however, is contracted largely from the rabbit and it was shown by Francis[17] that the disease known in Utah as deer fly fever is, in fact, tularemia transmitted from infected rabbits to man by the bite of the fly *Chrysops discalis*. Francis also found[18] that *Past. tularensis* was present in rabbits sold in the markets of Washington, D. C., and that a disease known as rabbit fever was not infrequent among those in contact with the rabbits. In 1938 2088 cases with 139 deaths and in 1939 2200 cases with 150 deaths were reported.

[13] Pirie and Grasset: South African Med. Jour., 1938, *12:*294; *ibid.*, 1941, *15:*275.

[14] Grasset: South African Med. Jour., 1941, *15:*373; Trans. Roy. Soc. Trop. Med. Hyg., 1942, *35:*203.

[15] *Cf.* Otten: Mededeel Dienst Volksgezondheid Nederland-Indië, 1941, *30:*61.

[16] McCoy and Chapin: Jour. Inf. Dis., 1912, *10:*61.

[17] Francis: Pub. Health Repts., 1919, *34:*2061.

[18] *Ibid.*, 1923, *38:*1391, 1396.

Human cases have been observed in all 48 States and in the District of Columbia.[19]

Morphology and Staining.—In culture *Past. tularensis* is a minute, gram-negative pleomorphic rod 0.2 μ in breadth and 0.3 to 0.7 μ in length; the coccoid form predominates in young cultures and the bacillary form in older cultures. In smears from the spleens of infected mice or guinea pigs the bacteria appear as coccoid forms in well-defined clusters. Capsules are present in the body; spores are not formed, and the microorganisms are non-motile.

On semisolid media *Past. tularensis* forms minute, transparent, droplike colonies that are mucoid in consistency and readily emulsifiable.

This bacterium is somewhat difficult to stain; methylene blue is not satisfactory, but either carbol fuchsin or aniline gentian violet may be used. Bipolar staining may be observed.

Physiology.—*Past. tularensis* differs sharply from the other members of this genus in that it will not grow on the ordinary media. It may be cul-

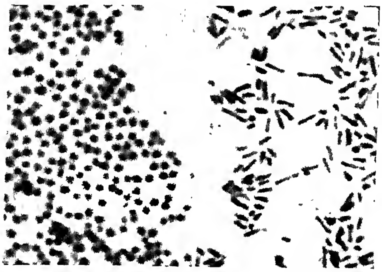


Fig. 90.—*Pasteurella tularensis.* Note change from coccoidal to bacillary form in twenty-four hours on fresh culture medium (Francis).

tured on a coagulated egg-yolk medium or on blood dextrose cystine agar.[20] Until recently it has been assumed that this bacterium could not be grown on a liquid medium. Tamura and Gibby[21] have found, however, that growth occurs in casein or gelatin hydrolysate medium supplemented with biotin, blood cell extract and liver extract. Steinhaus and McKee[22] have found that a medium of heart infusion, dextrose, cystine and hemoglobin will support good growth. It is aerobic and facultatively anaerobic, and its optimum temperature is 37° C. Fermentation reactions have been investigated in some detail by Francis[23] who has found that glucose, maltose and mannose are fermented, the fermentation of glycerol, levulose and dextrin is irregular, and mannitol, galactose, xylose, trehalose, salicin, arabinose, adonite, sucrose, lactose, amygdalin, dulcitol, erythritol, inosite, inulin, raffinose, sorbitol and rhamnose are not fermented. Differential

[19] *Cf.* Pub. Health Repts., 1940, *55*:667.
[20] Francis: Bull. No. 130, Hyg. Lab., 1922.
[21] Tamura and Gibby: Jour. Bact., 1943, *45*:361.
[22] Steinhaus and McKee: Pub. Health Repts., 1944, *59*:78.
[23] Francis: Jour. Bact., 1942, *43*:343.

fermentations are of no value. It is killed by exposure to 56° C. for ten minutes. It has been reported[24] that this bacillus contains an endotoxin.

Pathogenicity.—Two clinical types of tularemia are recognized, one the glandular or ulceroglandular type, which is the more common, the other the so-called "typhoidal" type. In the first instance the acute stage of the disease is characterized by headache, pains and fever, and a papule appears, frequently on a finger where presumably the bacilli enter the body, which later breaks down and forms an ulcer. The axillary and epitrochlear glands become painful and swollen and may break down with the discharge of purulent material. In cases infected via the conjunctiva, ulcers form on the inner surfaces of the eyelids and the cervical and pre-auricular glands may become tender and somewhat swollen. In the typhoidal type of the disease there are no local symptoms.

During the first week of illness the bacilli may be present in the blood and have been cultured, although in general cultures made directly from man are not successful and the bacillus is best isolated by guinea-pig inocu-

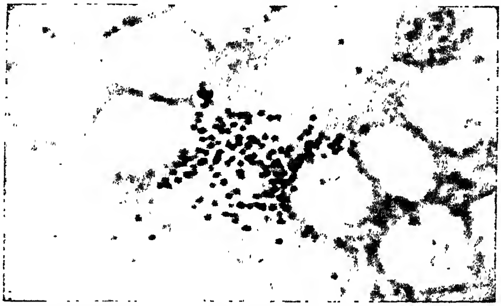


Fig. 91.—*Pasteurella tularensis;* coccoidal form; blood of rabbit (Francis).

lation and culture from necrotic foci found in liver, spleen and lungs of the pig on autopsy. Direct cultivation from the blood is rarely possible but has been accomplished, and it has been suggested that during the first week of the disease an initial bacteremia may occur which, in fulminating cases, develops into a septicemia.[25] The bacilli can only rarely be found in smear preparations from human cases. In the experimental disease the bacilli are present in the lymph spaces and phagocytic cells of infected tissues, and their presence within the cells in enormous numbers has caused some workers to suggest an intracellular proliferation of the microorganisms similar to that of the *Rickettsiae* (Chapter 35). Agglutinins are present in the blood in the second week of the disease and may persist in diminishing amounts for at least as long as eighteen years after recovery. The average duration of the disease is two to four weeks. The case fatality is low, 4.8 per cent and the pathology of the disease in man is not well known.[26]

[24] Oz: Science, 1940, *92:*113; original paper in Türkische Ztschr. f. Hyg. u. Exp. Biol., 1940, *2:*191.

[25] Ransmeier and Schaub: Arch. Int. Med., 1941, *68:*747.

[26] For postmortem findings *cf.* Mathews: New Orleans Med. and Surg. Jour., 1938, *90:*479.

A variety of lower animals have been found to be naturally infected—in addition to the ground squirrels and rabbits noted above, wild rats and mice, woodchucks, opossums, beavers, coyotes, deer, red foxes, ground hogs, muskrats, hogs, skunks, dogs, cats and lambs. The infection also occurs naturally in some birds such as sage hens, grouse and quail. The guinea pig is highly susceptible to artificial inoculation.

Epidemiology.—As indicated above, tularemia is acquired by man from lower animals either directly or indirectly. The bacilli may enter the unbroken skin of the guinea pig, and possibly this may occur in man through the dressing of infected rabbits and other animals, or the microorganisms may enter by means of minute abrasions on the skin of the hand. Eye infection occurs not infrequently; in fact, such infections were the first human infections with the bacillus observed.[27] Over 90 per cent of the human cases in this country are contracted from rabbits and it is estimated that about 1 per cent of wild rabbits are infected. Laboratory infection is

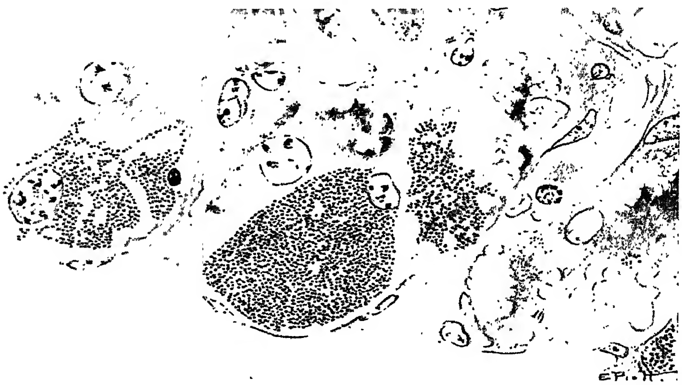


Fig. 92.—*Pasteurella tularensis* in hepatic cells of mouse (Francis).

not uncommon; 56 cases acquired from dissection were reported to 1940. *Past. tularensis* has also been found in streams[28] and is perhaps associated with the epizootics occasionally observed in beavers.[29] Water-borne epidemics have occurred in Russia and Turkey but none has been reported in this country.

The transmission of tularemia by an insect vector is common. In addition to the deer fly, *Chrysops discalis*, *Dermacentor andersoni*, *D. variabilis*, *D. occidentalis*, *Hemaphysalis leporis palustris*, *H. cinnabarina* and *Ixodes ricinus californicus* may carry it. In all probability the wood ticks serve to disseminate the infection in the animal population, and it is of particular interest that the infection is transmitted from the adult tick to the egg and both the larvae and the nymphs are infectious. Tularemia may, then, be in part maintained in the insect population.

[27] Wherry and Lamb: Jour. Inf. Dis., 1914, *15*:331.
[28] Pub. Health Repts., 1940, *55*:227.
[29] *Cf.* Jellison, Kohls, Butler and Weaver: Amer. Jour. Hyg., 1942, *36*:168.

Tularemia has been found in all parts of the United States, in Japan and in Central Europe,[30] and extensive epidemics have occurred in Russia.

Bacteriological Diagnosis of Tularemia.—As indicated above, *Past. tularensis* is difficult to cultivate from infected material. Specimens should be streaked on blood-dextrose-cystine agar. Characteristic minute, drop-like colonies may appear in three to five days but a culture should not be recorded as negative in less than three weeks. The procedure of choice is the intraperitoneal inoculation of a guinea pig with a saline emulsion of the specimen; relatively large amounts are required, usually 4 to 8 cc., of a fairly heavy emulsion. The animal should die in five to ten days. The pathology is characteristic and includes a hemorrhagic edema without pus at the site of inoculation, enlargement of the cervical, axillary and inguinal lymphatics which contain dry caseous material, and small white necrotic areas in the liver and spleen. Smears and cultures may be made but not infrequently the bacilli cannot be found or cultivated. In such instances the diagnosis depends on the guinea pig pathology.

Isolated cultures may be identified by specific agglutination. Conversely, patient's serum may be tested for agglutinins; a titer of 1:80 or higher is usually regarded as diagnostic if there are no *Brucella* agglutinins. In the event that *Brucella* is also agglutinated, the agglutination of *Past. tularensis* occurs more rapidly and to higher titer in tularemia.

Immunity.—An attack of tularemia confers a solid immunity, and second infections, when they occur, produce only a local lesion. It may be noted that antibodies (agglutinins) to *Past. tularensis* show some cross-reaction with *Brucella melitensis* and *Brucella abortus*, and for this reason, together with the complex nutritive requirements of these bacilli, some workers have placed them with the *Brucella* group and termed them *Br. tularensis*.

Prophylactic inoculation with vaccines is not successful in experimental animals in that it does not protect against the injection of virulent strains. There is some evidence, however, that vaccine prophylaxis in man confers a useful degree of protection.[31] The value of therapeutic antisera is doubtful; its use has been advocated by some,[32] but others[33] regard it as having no significant effect.

[30] Perm. Com. Office Int. Hyg. Pub., Session, May, 1937. *Cf.* Bull. Hyg., 1937, *12:*675.

[31] Foshay, Hesselbrock, Wittenberg and Rodenberg: Amer. Jour. Pub. Health, 1942, *32:*1131.

[32] Foshay: Medicine, 1940, *19:*1.

[33] Francis and Felton: Pub. Health Repts., 1942, *57:*44.

CHAPTER 25

THE HEMOPHILIC BACTERIA

Family: *Parvobacteriaceae*. Tribe: *Hemophileae*. Genus: *Hemophilus*. Minute rod-shaped cells, sometimes thread-forming and pleomorphic, non-motile, non-spore-forming, gram-negative. Strict parasites, growing best (or only) in the presence of hemoglobin, and in general requiring blood serum or ascitic fluid. Type species: *Hemophilus influenzae*.

The genus *Hemophilus* as at present constituted is a heterogeneous one. The true hemophilic or hemoglobinophilic bacteria are those whose growth necessitates or is especially favored by the presence of hemoglobin in the culture medium. The inclusion of bacteria other than these nutritionally distinctive types in the genus *Hemophilus* is questioned by many. Bacteria such as the bacillus of whooping cough and the Morax-Axenfeld and Ducrey bacilli are, then, to be associated with the truly hemophilic forms such as Pfeiffer's bacillus in only a tentative way pending a more satisfactory grouping.

THE HEMOPHILIC GROUP

Species	Growth Requirements		Hemolysis
	X Factor	V Factor	
H. influenzae	+	+	−
H. hemolyticus	+	+	+
H. parainfluenzae	−	+	±
H. suis (*influenzae suis*)	+	+	−
H. canis (*hemoglobinophilus*)	+	−	−
H. pertussis	−	−	+
H. duplex (Morax-Axenfeld)	−	−	±
H. ducreyi	−	−	+

HEMOPHILUS INFLUENZAE (PFEIFFER'S BACILLUS)

Hemophilus influenzae was isolated by Pfeiffer in 1892,[1] and was until relatively recently regarded by many as the etiologic agent of epidemic influenza. Influenza has, however, been shown to be caused by a filterable virus (p. 808), and the name *influenzae* has no etiological significance.

[1] Pfeiffer: Deut. med. Wchnschr., 1892, *18*:28; Ztschr. f. Hyg. u. Infektionskr., 1893, *13*:357.

Morphology and Staining.—Pfeiffer's bacillus is one of the smallest known pathogenic bacteria, rarely exceeding 1.5 μ in length and 0.3 μ in thickness. The ends of the cell are rounded, capsules are not generally observed but are present in smooth cultures, spores are not formed, and the bacillus is non-motile. There is a marked tendency to produce threads and other anomalous forms in culture which is, to some degree, a characteristic of strains. Some workers have attempted to differentiate varieties on the basis of morphology, but there is a continuous series of types, ranging from predominantly coccobacillary forms to predominantly longer bacilli and threads, and no sharp distinction can be made. There is a tendency, however, to regard the coccobacillary forms as "typical" and the longer forms as "atypical"; the typical form appears to predominate in strains isolated from pathological processes.

Fig. 93.—*Hemophilus influenzae*, pure culture. Note the variability from coccoid to bacillary form and the presence of longer filaments. Fuchsin; × 1050.

On blood agar the colonies of Pfeiffer's bacillus are very small, rounded, discrete and transparent and may reach the size of a small pinhead. If the culture is contaminated with other microorganisms, especially *Staphylococcus aureus*, the colonies are considerably larger, more opaque, and of a grayish white color, and develop most luxuriantly in the neighborhood of the foreign colony, a phenomenon termed the "satellite phenomenon."

These bacilli are somewhat more difficult to stain than most bacteria; Löffler's methylene blue for five minutes or dilute (1:10) carbol fuchsin for ten minutes is satisfactory. They are gram-negative.

Physiology.—One of the more fastidious bacteria, Pfeiffer's bacillus requires, as noted above, the presence of blood in the culture medium. It has been found that two substances present in such blood media are necessary to the growth of this bacterium; one, designated as the "X factor," is heat-stable and associated with hemoglobin, and the other the "V factor," is heat-labile and is found in yeast and various vegetable extracts as well as in whole blood. The satellite phenomenon noted above is due to

the formation of the V factor by other bacteria and its diffusion into the medium from the colony. The X factor is replaceable by hemoglobin or hematin. It seems that these or related compounds are required for respiration—some strains do not require it for anaerobic growth. It has been suggested that hematin is required for the synthesis of catalase; hematin can be replaced by cysteine which would reduce peroxide and make catalase unnecessary. The V factor may be replaced by coenzyme I or coenzyme II but not by nicotinic acid or its amide. Apparently the whole coenzyme molecule must be supplied and it is assumed that the thermolabile substance is of this nature (see p. 123).

A number of media have been devised for the cultivation of the influenza bacillus. Avery's oleate hemoglobin agar,[2] prepared by the addition of sodium oleate and a suspension of erythrocytes to an infusion agar base is one of the best of these, for not only is the growth of *H. influenzae* enhanced but the growth of streptococci and pneumococci and some other bacteria present in the sputum and nasal mucus is inhibited. The influenza bacillus grows luxuriantly on chocolate agar, prepared by the addition of fresh blood to hot (90° C.) infusion agar, and heavy growths for agglutination and other purposes may be obtained, but this medium does not differentiate and hence is not especially suitable for primary isolation. Fildes' agar,[3] an infusion agar base to which has been added a peptic digest of blood, supports good growth of the influenza bacillus and is used especially by British workers.

No growth occurs on gelatin or potato. Milk, containing blood, is rendered slightly alkaline by some strains. Nitrate is reduced to nitrite. Some strains—about 50 per cent—form indol. Fermentation reactions are variable, some strains being inactive while others ferment dextrose and other carbohydrates. Some strains are hemolytic while others are not. The hemolytic strains of *H. influenzae* do not appear to be clearly marked from the non-hemolytic strains by any other differential characters, since indol production and carbohydrate fermentation occur in both groups.

The influenza bacillus shows little resistance toward external conditions. Desiccation is quickly fatal. A pure culture suspended in water and then dried on silk threads loses its vitality within twenty-four hours; in dried sputum life is maintained somewhat longer, but not, as a rule, beyond forty-eight hours. The bacilli are readily killed by disinfectants. Even under favorable conditions artificial cultures soon die out, and in order to preserve vitality subcultures must be made every four or five days; chocolate agar is suitable for maintaining stock cultures.

Varieties.—Subdivisions of *H. influenzae* have been made by a number of workers, but whether such varieties deserve the dignity of species standing accorded some of them by the Bergey (1939) classification is open to serious question. The morphologically "typical" and "atypical" forms have been noted above, but these are not regarded as separate species. Other distinctions which have been made on the basis of hemolysis and nutritive requirements may be summarized briefly:

[2] Avery: Jour. Amer. Med. Assn., 1918, *71*:2050.
[3] Fildes: Brit. Jour. Exp. Path., 1928, *1*:129.

(1) The hemolytic and non-hemolytic varieties. The non-hemolytic form is designated *H. influenzae* by Bergey and the hemolytic form *H. hemolyticus*. Both require the X and V nutritive factors. These are generally regarded as a single species, *H. influenzae*.

(2) The swine influenza bacillus, *H. influenzae suis* or *H. suis*, which closely resembles Pfeiffer's bacillus except that it is relatively inert biochemically and differs immunologically. This bacterium, in association with a filterable virus, is causally associated with swine influenza. Both X and V factors are required for growth.

(3) The para-influenza bacilli, *H. parainfluenzae*, which closely resemble *H. influenzae* except that only the V factor is required for growth and these bacteria may be cultivated on agar containing serum or ascitic fluid. Although these bacilli are defined as non-hemolytic, hemolytic strains showing the same nutritive requirements are found.

(4) *H. canis* (*H. hemoglobinophilus*, *H. hemoglobinophilus canis*), found in the preputial secretions of dogs. It closely resembles *H. influenzae* except that it requires only the X factor for growth.

Variation and Antigenic Structure.—As tested by direct agglutination, *H. influenzae* is antigenically heterogeneous. Pittman,[4] however, has described rough and smooth forms in which the smooth form is encapsulated. By means of the agglutination test the encapsulated bacilli are found to fall into two immunological types, A and B, and the antigen responsible is a specific polysaccharide.[5] It appears that the majority of strains isolated are in the rough form, and these, by the agglutination test, are immunologically heterogeneous. More recent work,[6] however, has indicated that antigenic proteins may be extracted from the cell substance of influenza bacilli. One of these, fraction M, was found to be common to most strains of *H. influenzae*, indicating an immunological homogeneity demonstrable by precipitin tests with appropriately prepared antigens. It will be clear that the antigenic structure of *H. influenzae* is by no means fully understood as yet. The influenza bacilli appear to be immunologically related to certain of the pneumococcus types; Type A shows cross-reactions with pneumococcus Type VIb[7] and Type B cross reacts with pneumococcus Type VI and Type XXIX.[8] The immunological relationship of other *Hemophilus* species to the influenza bacillus and to one another is not yet known.

The S-R dissociation noted by Pittman is to some degree reversible by growth in the presence of anti-R immune serum. The relation of virulence to this dissociative change is not known.

Toxins.—As in the case of many other bacteria, the cell substance of the influenza bacillus is toxic to experimental animals, mice in particular, upon parenteral inoculation. Toxic substances are produced in fluid cultures, are filterable, and may appear in appreciable quantities after six to eight hours' incubation. Relatively large quantities of the filtrate (2 to

[4] Pittman: Jour. Exp. Med., 1931, *53*:471.

[5] *Cf.* Dingle and Fothergill: Jour. Immunol., 1939, *37*:53.

[6] Platt: Australian Jour. Exp. Biol. Med., 1939, *17*:19.

[7] Chapman and Osborn: Jour. Bact., 1942, *44*:620.

[8] Neter: Proc. Soc. Exp. Biol. Med., 1943, *52*:289; Zepp and Hodges: *ibid.*, 1943, *52*:315.

4 cc.), however, are necessary to produce death in rabbits, and it is probable that a true exotoxin is not formed.

Pathogenicity for Man.—The pathogenicity of Pfeiffer's bacillus for man is shown by the occurrence of cases of meningitis, mostly in infants and of a high fatality, in which *H. influenzae* is found in pure culture in the cerebrospinal fluid. These cases of "influenzal meningitis," while not very numerous, show that certain strains of this microorganism possess a definite invasive power. *H. influenzae* meningitis is the fourth commonest form of purulent meningitis; it occurs most often in the second six months of life and the case fatality rate is between 90 and 100 per cent.[9] Occasional cases of otitis media, appendicitis, sinusitis, and other localized infections may be caused by this bacterium.

In infections of the respiratory tract the influenza bacillus is frequently present, and it is found on autopsy in pneumonic lesions under conditions where its destructive action upon the tissues can hardly be doubted. Whether it is present as a primary or secondary invader in these cases is more uncertain. Its common occurrence in diseases like measles, whooping cough and tuberculosis indicates that its growth on human tissue is favored by the presence of other infecting agents. It seems probable that in respiratory infections *H. influenzae* commonly follows in the wake of some other microorganism.

It may be noted that the relation of this bacillus to influenza is very likely that of a secondary invader to the initial virus infection. It is frequently but not invariably present in cases of influenza and, of course, occurs in the absence of this disease. In the case of swine influenza, however, the influenza bacillus is, in association with a filterable virus, causally related to the disease (p. 809).

Pathogenicity for Lower Animals.—Except in swine influenza, the influenza bacillus is probably not a natural pathogen of lower animals. Upon intraperitoneal inoculation into laboratory animals, mice, guinea pigs and rabbits, large doses of these bacteria produce death within one or two days. Whether this is an actual invasion rather than a toxemia is uncertain; the bacilli may be found in the peritoneal exudate but usually not in the heart's blood, and petechial hemorrhages may be observed scattered over the peritoneum and, sometimes, the pleura. Certain strains produce a fatal infection in mice on intracerebral inoculation. As tested by intraperitoneal inoculation, the virulence of *H. influenzae* varies greatly from strain to strain; the virulent strains are in a minority and strains from influenzal meningitis are generally among the more virulent.

By inoculation of the mucous membrane of the upper respiratory tract of normal monkeys Blake and Cecil[10] succeeded in producing an acute upper respiratory disease resembling influenza with pathologic changes similar to spontaneous, uncomplicated *H. influenzae* pneumonia in man. Inoculation experiments upon man with pure cultures of these bacilli, however, have given a surprisingly large number of negative results.[11]

[9] *Cf.* Mutch: Lancet, 1941, *i*:751.
[10] Blake and Cecil: Jour. Exp. Med., 1920, *32*:691, 719.
[11] *Cf.* Rosenau: Jour. Amer. Med. Assn., 1919, *73*:311; McCoy and Richey: Pub. Health Repts., 1919, *34*:33.

Other hemophilic bacteria have been described in connection with diseases of lower animals. *H. canis*, for example, has been found in association with inflammation of the prepuce in dogs but is apparently harmless. Other hemophilic bacilli such as *H. bovis*, *H. gallinarum*, *H. muris*, *H. ovis* have been isolated from lower animals. Their relationship to *H. influenzae* and other better known species is not clear.

THE KOCH-WEEKS BACILLUS

A small bacillus, first observed by Koch[12] in 1883 in a series of eye inflammations in Egypt, was successfully cultivated by Weeks[13] in New York in 1887, and is now recognized as the cause of a world-wide and highly contagious form of conjunctivitis sometimes known as pink-eye.

It has been stated that the Koch-Weeks bacillus will grow on serum agar, or a mixture of glycerol agar and ascitic fluid or, at times, even on

Fig. 94.—Koch-Weeks bacillus in conjunctivitis; × 900 (Axenfeld, Kolle and Wassermann).

nutrient agar.[14] Others[15] have found, however, that both the X and V factors required by *H. influenzae* are required by the Koch-Weeks bacillus. It is, in fact, highly probable that this bacillus is identical with *H. influenzae*.[16]

The bacilli possess slight powers of resistance, and it is unlikely that dust is a means of conveying the infection. Direct contact with infective material through the medium of hands, towels, handkerchiefs, etc., is the usual mode of transmission. A peculiar form of hand infection due to an organism probably identical with the Koch-Weeks bacillus has been described by McDill and Wherry.[17]

[12] Koch: Arb. a. d. k. Gesund., 1887, 3 Auflage, 62.
[13] Weeks: Med. Rec., 1887, *31*:571.
[14] *Cf.* Kristensen: *Hemoglobinophilic Bacteria*. Copenhagen. 1922.
[15] Fildes: Brit. Jour. Exp. Path., 1923, *4*:265; Knorr: Centralbl. f. Bakt., 1924, *92*, 371, 385.
[16] Knorr: Deut. med. Wchnschr., 1925, *51*:65.
[17] McDill and Wherry: Jour. Inf. Dis., 1904, *1*:58.

BACILLUS PERTUSSIS (HEMOPHILUS PERTUSSIS)

Bacilli resembling *H. influenzae* were reported by early observers as occurring in a large proportion of cases of whooping cough. Although there are minor differences in the descriptions of these organisms as given by different observers, the cultural and morphological characters are essentially similar, and there seems little doubt that Spengler, Jochmann and Kraus, Wollstein, and Davis[18] discovered the same bacillus. More definite results were obtained by Bordet and Gengou,[19] who found in the bronchial exudate from cases of whooping cough a characteristic short oval bacillus which grew feebly on a special medium they devised. Earlier named *Bacillus pertussis*, this microorganism is now known either by that name or as *Hemophilus pertussis* or, more casually, as the Bordet-Gengou bacillus.

Morphology and Staining.—The Bordet-Gengou bacillus is a small ovoid rod from 1.0 to 1.5 μ in length and 0.3 to 0.5 μ in breadth. The

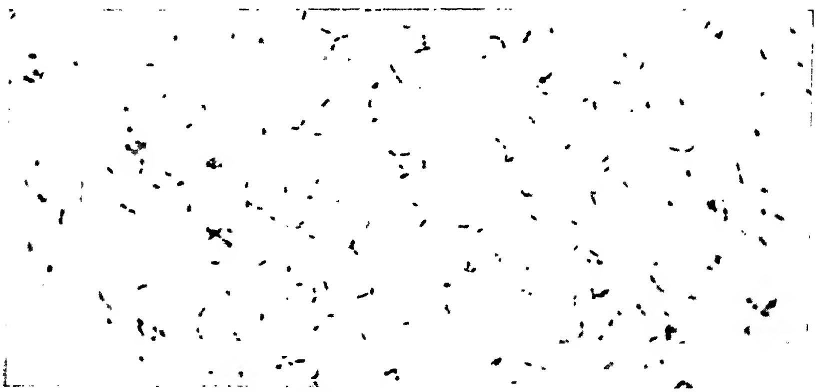


Fig. 95.—*Bacillus pertussis*, pure culture. Fuchsin; × 1050.

majority of the bacteria occur singly, although they may occasionally be seen in pairs end to end; chains do not occur in smears of bronchial exudate, but short chains may be seen in cultures in liquid media. This morphology is relatively constant and there is not the tendency to the formation of thread-like and other aberrant forms which is exhibited by the influenza bacillus. *B. pertussis* is non-motile and non-spore-forming, and the smooth form is encapsulated.

On the Bordet-Gengou medium the colonies are smooth, raised and glistening, with a metallic or pearl-like luster, and are larger and more opaque than those of the influenza bacillus. Forty-eight to seventy-two hours' incubation is required for their appearance and development. Upon further incubation they acquire a slight brownish color. A mucoid substance is abundantly produced by the culture, and the growth is sticky and tena-

[18] Davis: Jour. Inf. Dis., 1906, *3*:1.
[19] Bordet and Gengou: Ann. Inst. Pasteur, 1906, *20*:731.

cious. On blood agar the colonies are surrounded by a narrow zone of hazy hemolysis.

The bacilli stain with a little difficulty and, as in the case of the influenza bacillus, methylene blue or dilute carbol fuchsin must be applied for five to ten minutes; carbol toluidine blue is recommended by some workers, and stains the bacilli a lilac color. A tendency to bipolar staining may be observed. They are gram-negative.

Physiology.—*B. pertussis* is difficult to cultivate upon primary isolation, and the Bordet-Gengou medium upon which it grows readily consists of 1 per cent glycerol agar or glycerol broth made with macerated potato and added to an equal volume of human or rabbit blood. By repeated passage on media containing less and less blood, however, it may be acclimatized and will, in time, grow, although sparsely, upon ordinary nutrient agar. It does not, therefore, require the V and X factors that are essential to the development of the hemophilic bacteria. The optimum temperature is 37° C., and the bacillus is aerobic and facultatively anaerobic.

B. pertussis is biochemically inactive. It does not form indol, does not reduce nitrates, and does not ferment any sugars. It is in part because of this inactivity and lack of the strict nutritive requirements that characterize the hemophilic bacteria that many prefer not to include it in the genus *Hemophilus*.

The resistance of *B. pertussis* to deleterious influences is feeble and of the same order as that of the influenza bacillus. It is killed by exposure to 55° C. for thirty minutes.

Toxins.—As in the case of Pfeiffer's bacillus, the cell substance of *B. pertussis* is toxic upon parenteral injection into experimental animals and to about the same degree. Recent work seems to indicate definitely that an endotoxin is present in these bacilli which may be separated as a watery extract of disintegrated cells. There appear to be two fractions, one heat-stable and the other heat-labile and inactivated in thirty minutes at 56° C.[20] On intravenous inoculation in rabbits the heat-stable fraction produces a hyperglycemia and the heat-labile fraction a hypoglycemia. Intratracheal inoculation in rabbits produces an edematous reaction followed by a lymphocytic infiltration about the blood vessels and bronchi which is reported to be similar to the changes produced in the lung in whooping cough. Extracellular toxin found in cultures of *B. pertussis* appears to be liberated endotoxin.

Variation and Antigenic Structure.—Unlike the influenza bacillus, *B. pertussis* is generally in the smooth state when isolated from the body on an optimal medium, and is immunologically homogeneous. Leslie and Gardner[21] have found that their newly isolated strains fell into four immunologic groups which they designated as Phases I, II, III and IV. Although not obviously rough, Phases III and IV are somewhat rougher in appearance and less stable in saline suspensions than the bacilli of Phase I. The

[20] Evans and Maitland: Jour. Path. Bact., 1937, *45*:715; Erich, Bondi, Mudd and Flosdorf: Amer. Jour. Med. Sci., 1942, *204*:530; Evans: Jour. Path. Bact., 1943, *55*:269: Sprunt and Martin: Amer. Jour. Path., 1943, *19*:255.

[21] Leslie and Gardner: Jour. Hyg., 1931, *31*:423.

S-R dissociation of *B. pertussis* occurs readily on culture on artificial media, even on blood agar, and it seems probable that these phases do not represent distinct immunological types but rather successive stages in the S-R transformation. The importance of the existence of the bacilli in Phase I for the preparation of vaccines and the like has been stressed by many workers in the last few years; it will be clear, however, that in all probability this is but another way of saying that the bacilli must be in the smooth state.

The S-R change, of which the above phases represent the early stages, proceeds to the obviously rough stage with consequent alterations in colonial morphology and loss of virulence.

As indicated elsewhere (p. 446), *B. pertussis* is immunologically related to *Brucella bronchiseptica*. Eldering and Kendrick[22] have described atypical bacilli isolated from a small proportion of cases of whooping cough which they designate *Bacillus para-pertussis*. These bacilli differ from *B. pertussis* in that they grow readily on ordinary nutrient agar upon isolation and produce an alkalinity in litmus milk in two to four days. Immunologically, they are related to both *B. pertussis* and *Br. bronchiseptica*.

Pathogenicity for Man.—Interest in *B. pertussis* has centered chiefly about the possible etiological rôle of this microorganism in whooping cough. Although this bacillus was early observed in the bronchial exudate, where it is present in enormous numbers in the early stages of the disease, failure to reproduce the disease in laboratory animals and other considerations led many to question the etiological significance of *B. pertussis*. For example, the presence of inclusion bodies (p. 784) observed by McCordock[23] has suggested a filterable virus etiology. At the present time, however, the chain of evidence formalized as Koch's postulates appears to be complete, and it may reasonably be concluded that *B. pertussis* is the causal agent of whooping cough.

The microorganism is constantly present in the disease, most frequently in the catarrhal stage which is known to be the most contagious, less frequently in the paroxysmal stage, and disappears during the decline, being rarely found after the fourth week of disease. It is not found in healthy persons who have not been in contact with whooping-cough patients, or in patients with infections of the upper respiratory tract other than whooping cough.[24] By the cough-plate diagnostic method devised by Chievitz and Meyer[25] *B. pertussis* has been isolated in 88 per cent of the cases of whooping cough examined by Danish workers and in 98 per cent by Sauer and Hambrecht[26] in this country. The cellular infiltration and necrosis of the bronchi seen at autopsy in cases of whooping cough have been reproduced in the chick embryo by Gallavan and Goodpasture;[27] an interstitial pneumonia with leucocytic infiltration about the vessels and bronchioles and mucous secretion on the bronchial epithelium has been produced by intratracheal

[22] Eldering and Kendrick: Jour. Bact., 1938, *35*:561.
[23] McCordock: Proc. Soc. Exp. Biol. Med., 1932, *29*:1288.
[24] Wilcox: Jour. Inf. Dis., 1934, *55*:199.
[25] Chievitz and Meyer: Ann. Inst. Pasteur, 1916, *30*:503.
[26] Sauer and Hambrecht: Jour. Amer. Med. Assn., 1930, *95*:263.
[27] Gallavan and Goodpasture: Amer. Jour. Path., 1937, *13*:927.

inoculation in the mouse[28] and in the rat by intranasal inoculation.[29] Rich[30] has produced symptoms similar to those of whooping cough in man by the inoculation of chimpanzees, and MacDonald and MacDonald[31] have recorded experimental pertussis in man.

Significant work upon the relation of *B. pertussis* to the characteristic manifestations of whooping cough has been carried out by Mallory and his co-workers.[32] The production of a mild toxin by the bacillus and the absorption of the toxin seem to be shown by the exudation of leucocytes into the lumen of the trachea and bronchi, by slight changes in the lymph nodules of the spleen, lymph nodes, and gastro-intestinal tract, by the occurrence of the well-known lymphocytosis of whooping cough, and by the production of the antibody which makes possible the specific complement-fixation reaction. Possibly the cilia are damaged by a toxin, but this is not certain. More important than toxic action seems to be the mechanical disturbance caused by the presence of the bacilli in the respiratory tract. By their presence in enormous numbers, dozens to a hundred or more between the cilia of a single cell (Fig. 96), they are thought to interfere seriously with the normal ciliary action. In consequence, the removal of secretions and inhaled

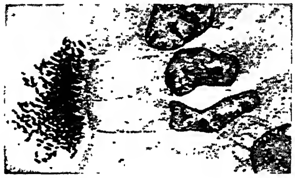


Fig. 96.—Whooping cough. Minute bacilli present in masses between cilia of two cells lining the trachea; × about 1500 (Mallory and Horner).

particles is prevented, and the lungs are probably more exposed to infection by inhalation than under ordinary circumstances. The bronchopneumonia which sometimes develops in fatal cases of whooping cough may be due to *B. pertussis* or to other bacteria, such as the pneumococcus.

Bacteriological Diagnosis of Whooping Cough.—The bacillus may be isolated by the cough plate method using the potato-glycerol-blood agar of Bordet and Gengou. The open plate is held 4 to 5 inches from the mouth and exposed to one or more explosive coughs. Characteristic colonies appear in two to three days but the plate should be retained for five days before discarding as negative. Nasopharyngeal swab cultures are reported to give a somewhat higher proportion of positive cultures than the cough-plate method.[33] The swab, on thin flexible copper wire, is passed through a nostril

[28] Burnet and Timmins: Brit. Jour. Exp. Path., 1937, *18*:83; Bradford: Amer. Jour. Path., 1938, *14*:377.

[29] Hornibrook and Asburn: Pub. Health Repts., 1939, *54*:439.

[30] Rich: Bull. Johns Hopkins Hosp., 1932, *51*:346; Rich *et al.*: Science, 1932, *76*:330.

[31] MacDonald and MacDonald: Jour. Inf. Dis., 1933, *53*:328.

[32] Mallory *et al.*: Jour. Med. Res., 1912–13, *22*:115, 391.

[33] Saito, Miller and Leach: Amer. Jour. Pub. Health, 1942, *32*:471; Miller, Leach, Saito and Humber: *ibid.*, 1943, *33*:839.

into the nasopharynx and left there for two or three coughs before withdrawal and culture on Bordet-Gengou medium. Sputum culture is not particularly satisfactory. Colonies of *B. pertussis* are larger and more opaque than those of the influenza bacillus; it may be differentiated further by hemolysis on blood agar and growth in the absence of the X and V factors.[34]

Epidemiology.—An upper respiratory infection, whooping cough is transmitted by means of the secretions of the mouth and nose, to some extent through towels, handkerchiefs, hand-to-hand contact and the like, and undoubtedly to a great extent by droplet infection. Undiagnosed and atypical cases may play an important part in the dissemination of the disease. The age incidence is marked with 96 per cent of the deaths in

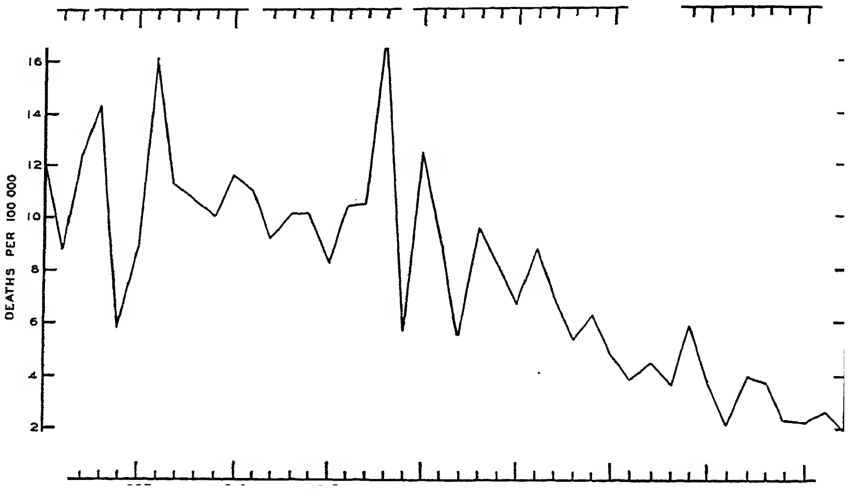


Fig. 97.—The prevalence of whooping cough in the Registration Area since 1900 as indicated by the uncorrected death rates. From data compiled by the Bureau of the Census.

children under five years of age. In 1940, for example, there were 2296 deaths from whooping cough in the registration area of the United States, a death rate more than triple that for the much more dreaded scarlet fever, and there is no doubt that whooping cough is one of the most important killing diseases of childhood. There appears to be some racial difference in susceptibility since the Negro death rate is considerably higher than that of the whites. In contrast to most other diseases the incidence in females is somewhat higher than that in males. The seasonal incidence shows a drop during late summer and early fall, with a prolonged peak in the later winter and spring (Fig. 98). The disease tends to recur in periodic epidemic waves,

[34] The laboratory diagnosis of whooping cough is discussed critically and in some detail by Donald: Brit. Med. Jour., 1938, Sept. 17, p. 613.

presumably a consequence of the accumulation of a new crop of susceptibles (Fig. 97).

Immunity.—Recovery from whooping cough is accompanied by the development of an immunity. Second attacks occur only infrequently in children and then are very mild; in older people second attacks are more severe. Complement-fixing antibodies are formed but do not appear until the third or fourth week of the disease and, consequently, are of limited diagnostic value. It has been claimed that immunes give an allergic skin reaction to the intradermal injection of pertussis bacilli; the value of this reaction is uncertain.[35]

Prophylactic Inoculation.—The early attempts to use vaccines of *B. pertussis* were not uniformly successful. More encouraging results were obtained by Madsen[36] and other Danish bacteriologists in the Faroe Islands. In the United States Sauer and his colleagues have prepared vaccines of freshly isolated, virulent strains which appear to be effective

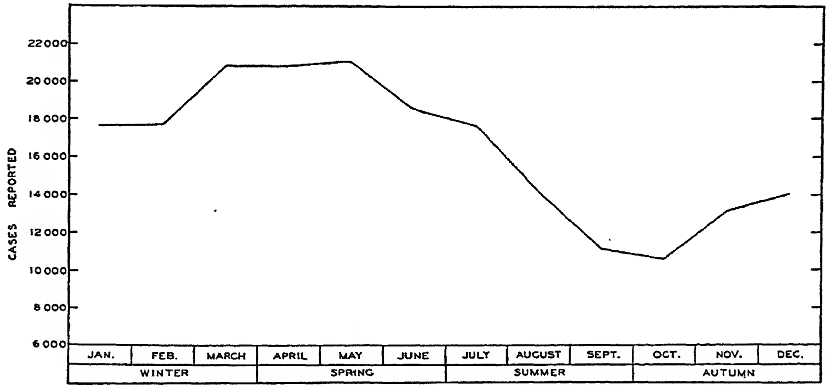


Fig. 98.—The seasonal incidence of whooping cough. Averages of reported cases by months for the years 1930 to 1938 inclusive. Data from Supplements to Public Health Reports.

in reducing the incidence and severity of the disease.[37] It appears to be of primary importance that the vaccine consist of the smooth bacilli of Phase I, and earlier reports of the inefficacy of active immunization are probably attributable in part, at least, to the use of other than the smooth form of the microorganism. It is now definitely established that active immunization with properly prepared vaccine confers an effective immunity to pertussis.[38] Alum precipitated vaccine seems to be effective

[35] *Cf.* Thompson: Jour. Hyg., 1938, *38*:104.

[36] Madsen: Jour. Amer. Med. Assn., 1933, *101*:187.

[37] Sauer and Hambrecht: Jour. Amer. Med. Assn., 1928, *91*:1861; Sauer: Jour. Amer. Med. Assn., 1933, *100*:239; *ibid.*, *101*:1449; *ibid.*, 1939, *112*:305.

[38] *Cf.* Kendrick and Eldering: Amer. Jour. Hyg., Sec. B, 1939, *29*:133; Maclean: Proc. Roy. Soc. Med., 1940, *33*:425; Joslin and Christensen: Amer. Jour. Dis. Children, 1940, *60*:1269; Perkins *et al.*: Amer. Jour. Pub. Health, 1942, *32*:63; Bustamante: Rev. Inst. Salubridad y Enfermedades Trop., 1942, *3*:189; Weiss and Kendrick: Amer. Jour. Hyg., 1943, *38*:306.

also but it is not known whether it is superior to plain vaccine.[39] It appears also that pertussis vaccine may be successfully combined with diphtheria toxoid to allow immunization against the two diseases simultaneously; available evidence suggests that the immune response is equal to that obtained with separate antigens.[40] It is generally agreed that the disease is appreciably milder in immunized children.

With the demonstration of the endotoxins of *B. pertussis*, considerable interest has attached to the possible rôle of anti-endotoxin in immunity to the disease. The toxicity appears to be neutralized with appropriately prepared antiserum and studies with experimental animals suggest that antitoxic antibody may be of importance in the immunity.[41] The results have not yet been applied to man except on a small scale.[42]

There is some evidence that antiserum may have prophylactic value[43] but neither antisera nor vaccines appear to have therapeutic value.

THE MORAX-AXENFELD DIPLOBACILLUS (HEMOPHILUS DUPLEX)

A small bacillus, described independently by Morax[44] and by Axenfeld,[45] is responsible for infections of the conjunctiva and cornea in man, and is known as the Morax-Axenfeld bacillus or *Bacillus lacunatus* (from the la-

Fig. 99.—Morax-Axenfeld diplobacillus; smear taken from conjunctiva (Brown Pusey).

cunae of liquefaction produced by growth on coagulated serum). It has been grouped with the hemophilic bacteria by Bergey as *Hemophilus duplex*.

The short rods, 1 μ by 2 to 3 μ, frequently appear end to end in pairs or short chains. They are non-motile, non-spore-forming and gram-negative. They do not grow on the ordinary nutrient media, potato, milk or gelatin

[39] Bell: Pub. Health Repts., 1941, *56:*1535.

[40] Schütze: Lancet, 1940, *ii:*192; Sauer and Tucker: Amer. Jour. Pub. Health, 1942, *32:*385; Kendrick: Amer. Jour. Hyg., 1943, *38:*193.

[41] See Evans: Lancet, 1942, *i:*529; Anderson and North: Australian Jour. Exp. Biol. Med. Sci., 1943, *21:*1; Roberts and Ospeck: Jour. Inf. Dis., 1944, *74:*14; Ospeck and Roberts: Jour. Inf. Dis., 1944, *74:*22.

[42] Bullowa and Alterman: Jour. Amer. Med. Assn., 1942, *120:*886.

[43] Bradford, Scherp and Brooks: Proc. Soc. Exp. Biol. Med., 1942, *49:*157.

[44] Morax: Ann. Inst. Pasteur, 1896, 10:337.

[45] Axenfeld: Centralbl. f. Bakt., I, 1897, *21:*1.

but require the presence of serum, ascitic fluid or blood in the culture medium. They ferment few if any carbohydrates and do not form indol. Coagulated serum is liquefied. They die out within a day or two at room temperature but may survive for weeks in culture in the incubator. It has been reported[46] that hemolytic and non-hemolytic types occur which may be differentiated immunologically.

So far as is known this microorganism is pathogenic only for the human eye. The inoculation of experimental animals is without effect, but the instillation of the bacilli onto the conjunctival sac of man results in the development of a blepharoconjunctivitis, either chronic or acute, and severe inflammation of the cornea may be produced. Treatment with 0.25 per cent zinc sulfate solution is specific and produces a rapid cure, while silver salts are without effect. The disease is widely distributed and has been reported in Europe, Africa and North America.

DUCREY'S BACILLUS (HEMOPHILUS DUCREYI)

"Soft chancre" or "chancroid" is a venereal disease transmitted by direct contact. The lesions, which are on the genitals or adjacent areas, are irregular ulcers which differ from the hard or hunterian chancre—the primary lesion of syphilis—in that they are not indurated. Unlike syphilis, the infection remains localized, spreading no further than the neighboring lymphatics, which may become swollen to form secondary buboes in the groin.

The bacillus which bears his name was found by Ducrey[47] in the purulent discharge from the lesion, and by the inoculation of the skin of the forearm he was able to transmit the disease through fifteen generations. The microorganism was obtained in pure culture by Besançon, Griffon and le Sourd[48] in 1890.

Ducrey's bacillus is a short rod 1 to 1.5 μ in length and 0.6 μ in breadth. In smears it is generally found to be ovoid, and there is a tendency to occur in end-to-end pairs or in short chains; in broth culture longer chains may be observed. It is non-spore-forming and nonmotile. The bacillus not infrequently stains irregularly and bipolar staining may be observed. It is stained by the usual aniline dyes and is gram-negative.

The bacillus will not grow on the ordinary laboratory media and requires the addition of serum or, preferably, blood. The small, grayish, glistening colonies appear in blood agar in twenty-four hours and, after two to three days' incubation, show a narrow zone of hemolysis. The bacilli may be cultivated from the chancroid by inoculating tubes of fresh (not more than three to five days old) rabbit blood. Smears made at the end of twenty-four to forty-eight hours' incubation will show the characteristic tangled chains of gram-negative bacilli.[49] These bacteria cannot be identified in smears from the chancroid because of a tendency to aberrant morphology.[50] The

[46] Oag: Jour. Path. Bact., 1942, *54*:128.
[47] Ducrey: Compt. Rend. Cong. Internat. Derm. Syph., 1890, 229.
[48] Besançon, Griffon and le Sourd: Presse med., 1890, *2*:385.
[49] Teague and Deibert: Jour. Urol., 1920, *4*:543.
[50] See the discussion of diagnosis by Greenwald: Jour. Amer. Med. Assn., 1943, *121*:9

bacilli may be isolated and cultivated on the chorioallantois of the developing chick embryo, and appear to have little pathogenicity for the embryo.[51]

There is little or no immunity. The chancroid is frequently multiple and is auto-inoculable. A hypersensitivity develops, however, which is manifested as a reaction to the intradermal inoculation of killed bacilli, and which persists for many years. Antisera and autogenous vaccines are said to have therapeutic value.

Ducrey's bacillus appears to be non-pathogenic for lower animals.

[51] Anderson and Snow: Amer. Jour. Path., 1940, *16*:269.

CHAPTER 26

PSEUDOMONAS; PROTEUS AND MORGAN'S BACILLUS; LACTOBACILLUS; LISTERELLA; NON-SPORE-FORMING ANAEROBIC BACILLI; BARTONELLA; PLEUROPNEUMONIA GROUP

PSEUDOMONAS PYOCYANEA

Family: *Pseudomonadaceae.* Tribe: *Pseudomonadeae.* Genus: *Pseudomonas.* Small, motile, aerobic, gram-negative rods with polar flagella. Commonly produce a water-soluble blue, green or yellow pigment. Powers of carbohydrate fermentation feeble. Widely distributed in water and soil. Some species are pathogenic. Type species: *Pseudomonas pyocyanea* (or *aeruginosa*).

The genus *Pseudomonas* includes some thirty species which are found, for the most part, in water, soil and wherever organic matter is decomposing. The fluorescent bacteria are members of this genus, *Ps. fluorescens* being the gelatin-liquefying form and *Ps. non-liquefaciens* the nonliquefying form. The elaboration of blue pigment by *Ps. syncyanea* results in "blue milk." One species, *Ps. septica*, is the cause of a disease of caterpillars, another, *Ps. jaegeri*, is pathogenic for chickens, and a third recently discovered species, *Ps. reptilovorus*, produces disease in certain reptiles.

The best known species of *Pseudomonas*, however, and the only one that is pathogenic for man, is *Ps. pyocyanea.* The blue or blue-green stains that sometimes appear upon surgical dressings long ago attracted attention, and even before the cause of the phenomenon had been discovered Fordos[1] studied the pigment. Gessard[2] found, in 1882, that the pigment was the product of a specific microorganism, *Ps. pyocyanea*, which he isolated in pure culture.

Morphology and Staining.—The cells of *Ps. pyocyanea* vary considerably in size and proportion, but appear usually as small, slender rods, 1.5 to 3 μ long and 0.5 μ broad, frequently united in pairs and short chains. There are 1 to 3 polar flagella, and the bacterium is actively motile. Neither capsules nor spores are formed. The colonies are large and spreading, edges are irregular, and the consistency butyrous. These bacilli stain readily with the usual aniline dyes and are gram-negative.

Physiology.—*Ps. pyocyanea* grows readily on all the ordinary culture media and most rapidly at a temperature of 30° to 37° C. Aerobic conditions are required, although it is sometimes said that there is some growth under anaerobic conditions. Gelatin is rapidly liquefied, hydrogen sulfide is produced, and indol formation is variable. The fermentative abilities of this bacterium are not great; acid is produced from dextrose, but most other

[1] Fordos: Compt. Rend. Acad. Sci., 1860, *51*:215.
[2] Gessard: *La pyocyanine.* These, Paris, 1882.

carbohydrates are not attacked. An alkaline reaction is produced in litmus milk; a soft coagulum is formed, followed by rapid peptonization and reduction of the indicator.

One of the most distinctive characteristics of *Ps. pyocyanea* is its production of a bluish green, soluble pigment which does not color the colonies or other masses of growth but instead diffuses into the medium. There are actually two pigments formed. The one, *pyocyanin*, is a deep blue in color and can be extracted from aqueous solution by chloroform, and the other, a yellowish green fluorescent pigment, is soluble in water but not in chloroform. They are both oxidation products of colorless precursors. Both sulfate and phosphate are required for the formation of pyocyanin. Pyocyanin is formed only by *Ps. pyocyanea*, but the fluorescent pigment is formed by several other species of *Pseudomonas*. These pigments may occur separately or together, and the conditions that determine their

Fig. 100.—Colonies of *Ps. fluorescens* on nutrient agar. Twenty-four-hour culture. × 3.

formation have been exhaustively studied.[3] Wrede[4] has determined the composition of pyocyanin, which has proved to be an entirely new type of dye and the first instance of a phenazine derivative occurring in nature (p. 129).

Pathogenicity.—For some time after its discovery *Ps. pyocyanea* was generally regarded as a harmless saprophyte, or at the most as a microorganism of slight pathogenic power. It has since been learned that this bacterium is causally associated with a great variety of suppurative and other affections in man. Apart from the many doubtful cases in which *Ps. pyocyanea* is found mixed with streptococci, staphylococci and other microorganisms where its share in inciting pathological processes is problematic, numerous instances are on record in which little or no question exists as to its etiological rôle. It has been found by a number of workers in pure culture in abscesses in different parts of the body, especially in the middle ear.

[3] Jordan: Jour. Exp. Med., 1899, *4*:627.
[4] Wrede: Ztschr. f. Hyg. u. Infektionskr., 1930, *111*:90.

Cases of endocarditis and pneumonia have also been met in which *Ps. pyocyanea* seemed to be the sole responsible microorganism. A generalized and fatal form of pyocyanic infection has been observed by a number of investigators, and the bacillus has been found in the blood during life. *Ps. pyocyanea* has been found constantly present in the intestinal discharges of patients during a dysentery-like epidemic, and the same microorganism was also present in abundance in drinking water which seemed, on epidemiological grounds, to be implicated in the outbreak. Ghosh[5] reported a series of cases of infection with this bacillus that closely simulated Asiatic cholera. There is no doubt that under certain conditions *Ps. pyocyanea* is pathogenic for man, although probably human infections are relatively rare.

The intraperitoneal injection of 0.25 cc. of culture of a virulent strain will kill a guinea pig with acute symptoms in twenty-four hours. Smaller amounts are also fatal but less rapidly. Subcutaneous inoculation produces a marked local reaction. The symptom-complex presents nothing especially characteristic. Rabbits are not so susceptible as guinea pigs; mice and pigeons are less susceptible than rabbits. Immunity can be produced by small, non-lethal doses.

The occurrence of *Ps. pyocyanea* as a plant pathogen and its close relationship, if not identity, with *Phytomonas polycolor*[6] has been noted elsewhere (p. 295).

PROTEUS

Family: *Enterobacteriaceae*. Tribe: *Proteae*. Genus: *Proteus*. Highly pleomorphic gram-negative rods. Actively motile with peritrichal flagella. Luxuriant growth on ordinary media with characteristic spreading colonies. Gelatin liquefied; dextrose and sucrose are fermented with gas production, but lactose is not attacked. Type species: *Proteus vulgaris*.

These microorganisms were originally described by Hauser[7] as an independent genus containing three species—*Pr. vulgaris*, *Pr. mirabilis*, and *Pr. zenkeri*. It is now generally agreed that the last is not closely related to the typical members of the group (it is gram-positive), and it is now placed in a separate genus as *Kurthia zenkeri*.[8] The genus *Proteus* now contains eight species, but the rapidity of gelatin liquefaction is no longer used as a differential character.[9]

Proteus (*vulgaris*) is one of the most common bacteria in soil and water containing decomposing organic matter of animal origin. It is often found abundantly in sewage, but does not occur in normal feces so frequently as sometimes stated. It is perhaps to be identified with "the bacterium of putrefaction," the so-called "*Bacterium termo*" of early writers.

[5] Ghosh: Jour. Indian Med. Assn., 1938, *7*:655.

[6] Elrod and Braun: Jour. Bact., 1942, *44*:633.

[7] Hauser: *Über Fäulnissbakterien*. Leipzig. 1885.

[8] As of the 1939 Bergey classification. Formerly *Zopfius zenkeri*, the genus *Zopfius* has now been discarded.

[9] Comprehensive studies of the *Proteus* group have been made by Bengtson: Jour. Inf. Dis., 1919, *24*:428; Wenner and Rettger: Jour. Bact., 1919, *4*:331; Moltke: *Contribution to the Characterization and Systematic Classification of Bact. proteus vulgaris* (*Hauser*). Copenhagen. 1927. See also the discussion of the taxonomic relationships in this group by Rustigan and Stuart: Proc. Soc. Exp. Biol. Med., 1943, *53*:241.

Morphology and Staining.—In general, these bacteria appear as straight or slightly curved rods 1 to 2.5 μ in length and 0.4 to 0.6 μ in breadth, frequently in end-to-end pairs and short chains. Ovoid forms are common, however, and long, curved, filamentous cells predominate in actively swarming cultures. *Proteus* is actively motile by peritrichous flagella and forms neither capsules nor spores.

The phenomenon of "swarming" exhibited by these bacilli is a consequence of their active motility. On the surface of agar media the colonies do not remain compact and discrete; the growth spreads rapidly over the entire surface available as a thin, scarcely visible bluish film. Microscopic observation shows that the bacilli break away from the edge of the growth and migrate or "swarm" over the surface of the medium, thus giving rise to the thin film of growth. This property is a source of considerable incon-

Fig. 101.—*Proteus vulgaris*. Smear from a pure culture. Note the coccobacillary form The occasional occurrence of paired cells is a result of active multiplication. Fuchsin; × 1050.

venience in the isolation of bacteria other than *Proteus* from mixed cultures in which it is present; the inclusion of 0.01 per cent sodium azide inhibits the growth of this and other gram-negative bacilli but allows the growth of streptococci.[10]

These bacilli stain readily with the usual aniline dyes and are gram-negative.

Physiology.—The nutritive requirements of *Proteus* are simple, and the bacilli grow readily upon the ordinary laboratory media. They may be cultivated in synthetic solutions containing ammonium lactate, but nicotinic acid must be supplied.[11] The optimum temperature for growth is 30° to 37° C., though good growth occurs at 20° C. These bacteria are facultative anaerobes, but anaerobic growth is generally very poor.

Gelatin is liquefied more or less rapidly, often with a characteristic colony formation with radiating filaments which wander far off into the surround-

[10] Snyder and Lichstein: Jour. Inf. Dis., 1940, *67*:113.
[11] Fildes: Brit. Jour. Exp. Path., 1938, *19*:239.

ing medium. The typical *Proteus* colonies are best formed when the gelatin is soft, as happens when it is kept at a temperature not far below the melting point or is made up with 5 instead of 10 per cent gelatin. Dextrose is fermented with acid and gas production; sucrose and maltose are fermented by some, but not by all, strains; lactose, raffinose and mannitol are never fermented by the typical *Pr. vulgaris*. The Voges-Proskauer reaction is negative and the methyl-red test positive. Nitrates are reduced. Milk is at first rendered slightly acid, then curdled with alkaline reaction (in about three days), and more or less slowly peptonized.

Moltke[9] found that production of hydrogen sulfide and the decomposition of urea distinguish *Proteus* from all other gram-negative gelatin-liquefying bacilli. In a study of 194 strains he observed a definite division on the basis of maltose fermentation; 37 fermented maltose and 157 did not. All but one of the maltose-positive strains produced indol and none of the maltose-negative did. There were other correlated qualities.

Proteus was long regarded as especially concerned in the putrefaction (anaerobic decomposition) of protein substances, but Rettger and Newell[12]

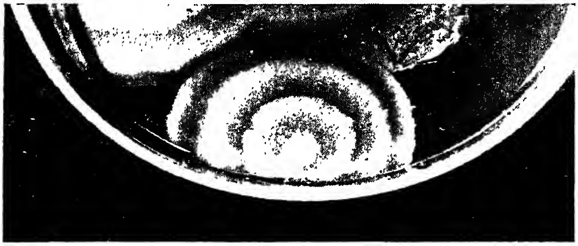


Fig. 102.—*Proteus vulgaris* colony on blood agar. Note the swarming exhibited as successive waves of growth (Dack).

have shown that no decomposition of protein is effected by *Proteus* under anaerobic conditions, but with aerobic growth the ordinary non-putrefactive products of protein decomposition are formed.

Antigenic Structure.—*Proteus* contains both *H* and *O* antigens (p. 269) when motile, and the non-motile, non-swarming strains contain only *O* antigen. According to Moltke,[9] the swarming strains can be divided into three groups by direct agglutination and into subgroups by absorption tests. Certain *Proteus* strains are agglutinated by the serum of patients having typhus fever. These so-called *X* strains contain an antigen common to the typhus *rickettsiae* and the agglutination of these bacilli (the Weil-Felix reaction) is of diagnostic value in typhus fever (p. 766). In the *Proteus* strains the antigen is a part of the *O* antigen and its specificity is determined by an alkali-stable carbohydrate haptene which is also found in the *Rickettsia prowazeki*.[13] The best known of the *X* strains is *X*-19. The *X* strains, it may be noted, frequently ferment maltose.

[12] Rettger and Newell: Jour. Biol. Chem., 1912, *13*:341.
[13] *Cf.* White: Brit. Jour. Exp. Path., 1933, *14*:145; Castaneda: Jour. Exp. Med., 1934, *60*:119: *ibid.*, 1935, *62*:289.

Pathogenicity.—*Proteus*, both in mixed and pure cultures, has been found to be associated with a variety of pathological conditions. Infections of the eye and ear, pleuritis and peritonitis and suppurative abscesses in many parts of the body are among the many instances in which the pathogenic power of this bacterium in pure culture can hardly be doubted. As a producer of cystitis it probably ranks next to *Bacterium coli.* Besides its independent pathogenicity, it is found so commonly associated with other microorganisms in purulent war wounds and similar processes that its activity as an accomplice is probably second only to that of the cocci.

In certain affections of the digestive tract *Proteus* has been frequently held to be the responsible agent. In diarrheic stools, especially those of infants, it has often been found in large numbers, and is regarded by many as a cause of infant diarrhea.[14] The real relation of *Proteus* to intestinal infection is, however, still quite obscure.

Certain food-poisoning epidemics have been ascribed to *Proteus.* These cases have been collected and subjected to a critical analysis by Bengtson,[15] who concludes that a definite proof of the causal relationship between the bacterium isolated and the illness is lacking in all instances. Further evidence is needed to establish the rôle of *Proteus* in food poisoning.

Animal inoculation shows that a great range of virulence exists among the *Proteus* cultures that have been tested. Freshly isolated strains from pathological sources may produce definite lesions, including abscesses, enlargement of the spleen and a diarrheic condition. One strain from a case of peritonitis has been reported which killed mice in amounts of 0.005 cc. of broth culture. The etiologic agent of "red-leg" disease of frogs, "ulcer disease" of brook trout, and "red sore" of pike has been found to be a bacillus closely related to the *Proteus* group and it has been classified as *Proteus hydrophilus*.[16] The cell substance of these bacteria is toxic on parenteral injection and, as in the case of the enteric forms, appears to be a glucolipoid.[17] No difference in toxicity is apparent between strains of pathogenic and non-pathogenic origin.

Morgan's Bacillus.—This bacillus was isolated by Morgan[18] from the stools of infants suffering from summer diarrhea. Although it was first designated as "Morgan's No. 1," the other varieties of "Morgan's bacilli" have long since faded out of general interest and recognition, so that the name Morgan bacillus is usually applied to "Morgan's No. 1." In its cultural characteristics Morgan's bacillus resembles the coliform bacteria and, since it does not ferment lactose, has been grouped by some with the paracolon bacilli.[19] Although it does not liquefy gelatin, more recent reconsiderations[20] suggest that it is more closely related to the *Proteus* group, and it is therefore classified as *Pr. morgani.*

[14] *Cf.* Neter and Farrar: Amer. Jour. Digest. Dis., 1943, *10*:344.
[15] Bengtson: Jour. Inf. Dis., 1919, *24*:428.
[16] Kulp and Borden: Jour. Bact., 1942, *44*:673; Reed and Toner: Canadian Jour. Res., Sec. C, 1942, *20*:161.
[17] Bachmann and Mielke: Centralbl. f. Bakt., I, 1939, *144*:268.
[18] Morgan: Brit. Med. Jour., 1906, *i*:908.
[19] Jordan, Crawford and McBroom: Jour. Bact., 1935, *29*:131.
[20] *Cf.* Rauss: Jour. Path. Bact., 1936, *42*:183; Sevin and Buttiaux: *ibid.*, 1939, *49*:457.

This bacillus appears to have played a part in a number of outbreaks of summer diarrhea of infants and has been isolated from paratyphoid-like fevers. It has been found to give rise to spontaneous epidemics of enteritis in mice. Injected intraperitoneally into mice, Morgan's bacillus produces a rapidly fatal infection.

LACTOBACILLUS*

This genus is composed of a somewhat loose assemblage of microorganisms which produce considerable quantities of lactic acid from carbohydrates and which are able to withstand a degree of acidity usually fatal to non-sporulating bacteria. The latter characteristic is useful in the isolation of cultures as well as in distinguishing the group. Kendall[21] has proposed a term, *aciduric* (acid-tolerant), which is now commonly applied.

Morphologically some of the lactobaccilli are long, slender rods, while others are somewhat similar to the colon bacillus but unlike it they are

Fig. 103.—*Lactobacillus* sp. isolated from the mouth. Morphologically identical with *L. acidophilus*. Note the diplobacillary form and palisade arrangement of the cells. × 2500 (R. W. Harrison).

all gram-positive. They are non-motile. Many cultures exhibit a typical diplobacillary form, often kidney-shaped. Old cultures frequently exhibit considerable pleomorphism. As a rule, cultures are facultatively anaerobic or microaerophilic, and some of them, particularly *L. bifidus*, are anaerobic on primary isolation.

Classification has been based primarily upon source of the cultures. Although this is an insecure criterion for differentiation, no really satisfactory basis of classification has been developed. Rahe[22] and a number of other investigators have attempted classifications by means of carbohydrate-fermentation reactions. Many strains otherwise apparently identical

* The section on *Lactobacillus* is written by Dr. R. W. Harrison, Dean of the Division of Biological Sciences, and Professor of Bacteriology, Zoller Memorial Dental Clinic, the University of Chicago.

[21] Kendall: Jour. Med. Res., 1910, *22*:153.

[22] Rahe: Jour. Inf. Dis., 1914, *15*:141; 1915, *16*:210.

differ, however, in fermentative reactions, and many cultures change in reaction after prolonged artificial cultivation. These variations make it doubtful whether such reactions are of any more value than morphological and colonial characters which are also subject to considerable variation. Efforts to classify the lactobacilli on the basis of metabolic activity have been more promising. Davis and Rogers[23] separated these organisms into three groups differing in respiratory mechanisms. Curran, Rogers and Whittier[24] had earlier investigated the possibility of differentiation by means of the end products of metabolism. The serological relationships of a number of species have been studied by several investigators. Agglutination tests, hampered often by spontaneous agglutination, have tended to complicate rather than simplify the problem. These reactions in some groups have been so heterogeneous as to indicate strain-specificity. In other cases close relationships have been demonstrated. For example, *L. bifidus* and *L. acidophilus* were found by Cruickshank[25] and by Weiss and Rettger[26] to be closely related. Harrison and co-workers, using strains of oral origin, studied their polysaccharide precipitation reactions in an effort to establish an immunological classification. They reported the existence of four or more immunological types[27] but indicated later that some strains are unstable, changes in immunological specificity occurring apparently in association with changes in fermentation reaction.[28] There is evidence[29] that cultures from the intestine include types identical with those obtained from the mouth.

About forty species are recognized, of which most have been isolated from milk products or fermented foods and are widely used in various industrial processes. Some have been obtained from soil, and others are common inhabitants of the alimentary canal. Interest in this group, except for its uses in industrial fermentation processes, has been directed largely toward those members associated with dental caries and the species found in the intestinal flora.

Lactobacillus Acidophilus.—First cultivated by Moro in 1900 from the feces of infants, this organism has been isolated from the intestine of nearly all the mammalia, many other vertebrates and some of the invertebrates. It increases in the intestine when the carbohydrate content of the diet is increased and may become predominant when a milk diet is administered.[30] These bacilli, fairly large and of variable length, are arranged singly, in pairs frequently slightly bent at the juncture, and in palisades (Fig. 103). Long chains, filamentous forms and club shapes are not uncommon. Young cultures stain uniformly gram-positive. Old cultures, how-

[23] Davis and Rogers: Chem. and Indust. (London), 1939, *58*:1021.
[24] Curran, Rogers and Whittier: Jour. Biol. Chem., 1919, *39*:347.
[25] Cruickshank: Jour. Hyg., 1925, *24*:241.
[26] Weiss and Rettger: Jour. Bact., 1934, *28*:501.
[27] Harrison, Zidek and Hemmens: Jour. Inf. Dis., 1939, *65*:255; Harrison: *ibid.*, 1942, *70*:69.
[28] Harrison: Jour. Inf. Dis., 1942, *70*:77.
[29] Harrison and Opal: Jour. Dent. Res., 1944, *23*:1.
[30] Rettger and Cheplin: *A Treatise on the Transformation of the Intestinal Flora with Special Reference to the Implantation of Bacillus Acidophilus.* New Haven. 1933.

ever, often show beading or bipolar staining and may be easily destained so that they appear to be gram-negative. Colonies, usually small, may vary in shape between a smooth, rounded opaque form and a flattened, translucent, irregular form often having a ground-glass appearance. Fermentation reactions are variable, but most strains produce acid, no gas, from glucose, lactose, maltose and sucrose, and coagulate milk within forty-eight hours. The organism is used in the preparation of acidophilus milk, a buttermilk with considerable acidity, for which a number of therapeutic claims have been made.[31] Döderlein's bacillus (1892), (*Bacillus vaginalis, Bacillus crassus*), a common constituent of the flora of the vagina and believed to aid in the natural defenses against infection by contributing to the acidity of the vaginal secretions, is thought to be identical with *L. acidophilus*.

Lactobacillus Bifidus.—Apparently closely related to *L. acidophilus* and often difficult to distinguish from it, *L. bifidus* is usually a thinner rod with ends somewhat more tapering and sometimes bifurcated (Fig. 104). It was isolated from feces of breast-fed infants by Tissier in 1900. Although

Fig. 104.—*Lactobacillus bifidus*. Note the Y-shaped forms (Dack).

common in the intestine of breast-fed infants, sometimes reaching over 90 per cent of the total intestinal flora, it is less conspicuous in the intestinal contents of bottle-fed babies. It is sometimes found also in the feces of adult animals, including man. Antigenically it is closely related to *L. acidophilus* and like it produces acid, mainly lactic, from a number of sugars. Unlike the latter, it usually ferments inulin. It is anaerobic on primary isolation and some strains never grow well under aerobic conditions. Growth is enhanced by cystine.[32] Partly because of its anaerobic requirements, it is sometimes classified with the *Bacteroides*. Tissier also isolated from feces of infants a similar organism (*Lactobacillus exilis*) which differs from the first in the possession of a more regular morphology and in greater facility of growth under aerobic conditions.

Lactobacillus Bulgaricus.—This name is given to an organism isolated by Grigoroff in 1905 from Bulgarian fermented milk. It gained promi-

[31] For an analysis of this subject see Rettger, Levy, Weinstein and Weiss: *Lactobacillus acidophilus and Its Therapeutic Application*. Yale University Press. New Haven. 1935.

[32] Blaurock: Zentralbl. f. Bakt., I Abt., 1939, *144*:75.

nence through the work of Metchnikoff, who believed that intestinal putrefaction could be restrained by drinking milk fermented by this organism. When it was later shown that *L. bulgaricus* does not become implanted in the intestine, its use in experimental therapeusis was dropped in favor of *L. acidophilus*. More difficult to cultivate than *L. acidophilus*, slightly larger and somewhat different in sugar fermentations, it is nevertheless closely related. Sherman and Hoge have reported[33] that *L. bulgaricus* rarely grows at 15° C., dies out on repeated culture in a lactose-peptone-yeast extract broth, is unable to grow in media containing 2.5 per cent NaCl and

Fig. 105.

Fig. 106.

Fig. 105.—Ground section of human tooth enamel showing an unstained bacterial plaque growing on the surface. Note the enamel rods and filamentous bacteria. × 1200 (Blayney).

Fig. 106.—Ground section through an early carious lesion in human tooth enamel stained with gentian violet. Note the intimate contact of the bacterial plaque with the eroding surface. × 500 (Blayney).

fails to grow in broth at *p*H 7.8, while *L. acidophilus* will grow under all these conditions. The Boas-Oppler bacillus, first seen in 1895 in the gastric juice of patients suffering from carcinoma of the stomach and cultivated by Heinemann and Ecker[34] from patients with this and other gastric diseases, is a member of this group, similar to, if not identical with, *L. bulgaricus*.

Dental Caries.—There is much disagreement concerning the basic factors responsible for susceptibility and resistance to dental decay, and there

[33] Sherman and Hoge: Jour. Bact., 1940, *40*:11.
[34] Heinemann and Ecker: Jour. Bact., 1916, *1*:435.

is a wide diversity of opinion as to the relative importance of general systemic conditions, diet, bacterial flora of the mouth and various local factors in the oral environment. Nevertheless, it has been well established that certain microorganisms, particularly members of the lactobacillus genus, are usually associated with the disease and, although there is not as yet direct experimental proof, considerable indirect evidence has been adduced to indicate that this association is etiological.[35] It has not been shown, however, that other carbohydrate-fermenting, acid-producing organisms are not implicated also.

Much of the evidence that lactobacilli are associated with dental caries has been obtained from cultures of saliva. Many investigators have found close correlation between the numbers of lactobacilli in saliva and the degree of carious involvement of the teeth. Such a correlation is by no means invariable, however. In some studies[36] lactobacilli have been found to be a minor constituent of the salivary flora and to be infrequent in or absent from the flora of carious cavities. Bradel and Blayney[37] in a study of some 4000 saliva samples from carious patients found in the majority of cases a fair degree of correlation between the numbers of lactobacilli and the amount of tooth decay. There were, however, more than 900 samples in which they did not find any lactobacilli. Bibby[38] has suggested that the association of lactobacilli with dental caries is a result of the favorable habitat furnished by the cavities in the teeth. A number of dental bacteriologists differ from this view and interpret the results of saliva cultures as indicating a correlation with the degree of caries "activity" rather than with the amount of cavitation present. A "caries activity test"[39] based on this hypothesis has been developed.

Most of the present-day theories of the etiology of dental caries, whether they assign primary or secondary rôles to the oral flora, are based on the hypothesis that the actual process of decalcification is due to the action of organic acids elaborated on the tooth by microorganisms. Areas of the tooth surface which are protected from the cleansing action of roughage in the diet are covered by bacterial plaques, thin, tenacious, felt-like layers composed of leptotrichia, actinomyces and a variety of other microorganisms including chiefly bacillary and coccal forms. A section of a tooth with a plaque attached is shown in Fig. 105. The underlying enamel is sound.

A relationship of the bacterial plaque to the initial stages of dental caries was first clearly postulated by Black[40] near the turn of the century but little experimental confirmation has been advanced until recently. After

[35] For reviews see Ch. VIII by T. Rosebury and Ch. X by P. Jay in *Dental Science and Dental Art*, edited by S. M. Gordon. Lea & Febiger. Philadelphia, 1938.

[36] *Cf.* Bibby, Volker and Van Kesteren: Jour. Dent. Res., 1942, *21:*61; Anderson and Rettger: *ibid.*, 1937, *16:*489.

[37] Bradel and Blayney: Jour. Amer. Dent. Assn., 1940, *27:*1601.

[38] Bibby: In *Dental Caries*. University of Pennsylvania Press. Philadelphia, 1941, pp. 27–43.

[39] *Cf.* Jay: Amer. Jour. Pub. Health, 1938, *28:*759.

[40] Black: *Operative Dentistry*. Medico-Dental Publishing Co. Chicago, 1908, Vol. 1, p. 75.

caries begins under a plaque it extends through the enamel as in the illustration in Fig. 106, and the dentin becomes involved. The lesion in the dentin extends laterally at the junction of enamel and dentin, and centrally as bacteria invade the dentinal tubules (Fig. 107). It eventually reaches the pulp, or so-called "nerve," and the tissues around the root may finally become infected.

While acid production by the microorganisms on the tooth is no doubt controlled to a considerable extent by the amount and nature of the food retained on the surface, dental caries does not occur in all food-retentive areas. Localization of the initial lesion, dependent partly perhaps upon a variety of local factors, is apparently determined primarily by the nature of the plaque. Although knowledge of the bacterial flora of the plaque is still incomplete, recent work indicates that certain types of lactobacilli,

Fig. 107.

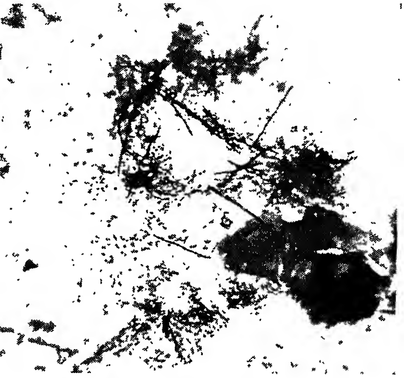


Fig. 108.

Fig. 107.—Ground section of carious human dentin showing bacteria in the dentinal tubules. × 1000 (Blayney).

Fig. 108.—Smear of material from a fragment of a bacterial plaque like that illustrated in Fig. 106. Note the bacillary forms which have been teased out of the filamentous masses. × 1000 (Blayney).

vigorous in acid production and highly acid-tolerant, are commonly present in plaques covering areas of beginning caries and are found infrequently in those removed from sound enamel surfaces. Stephan[40a] has found the reaction of plaques over caries-active areas to be pH 5.0 or less immediately following a glucose rinse. A smear from a typical carious plaque showing lactobacilli as well as a number of other forms of microorganisms is illustrated in Fig. 108. Although the basic bacterial flora of the plaque may vary considerably,[40b] the filamentous forms are apparently always present. Whether they play any rôle in the caries process other than that of entrapment and protection of other bacteria is not known.

[40a] Stephan: Jour. Dent. Res., 1944, *23*:257.

[40b] Hemmens, Blayney and Harrison: Jour. Dent. Res., 1941, *20*:29; 1943, *22*:205; Hemmens, Blayney, Harrison and Bradel: *ibid.*, 1943, *22*:223.

LISTERELLA

Family: *Bacteriaceae*. Genus: *Listerella*. Small, non-spore-forming, gram-positive rods, motile by means of a single terminal flagellum. Aerobic to microaerophilic. Growth occurs readily on ordinary media. Parasitic and pathogenic. There is only one species: *Listerella monocytogenes*.

A small gram-positive bacillus was isolated in 1926 by Murray, Webb and Swann[41] from rabbits having a disease characterized chiefly by a large mononuclear leucocytosis. It was originally named *Bacterium monocytogenes*, but is now generally known as *Listerella monocytogenes*[42] and infection with this bacterium is called *listerellosis*.

The microorganism is a small rod motile by means of a single polar flagellum and 0.5 μ in breadth and 1 to 2 μ in length. Neither spores nor

Fig. 109.—*Listerella monocytogenes*. Smear from a pure culture. Fuchsin; × 1050.

capsules have been observed. The cells occur singly, in V-shaped or parallel pairs, and in short chains. They usually stain uniformly with the ordinary aniline dyes, though sometimes the stained cells appear beaded. Colonies on semisolid media are small, transparent by transmitted light, and milk-white by reflected light. They are hemolytic on blood agar, and appear not unlike hemolytic streptococci or some of the diphtheroids.

Growth is best on enriched media such as liver extract and blood agar. The optimum temperature is 37° C. This bacterium is aerobic and facultatively anaerobic. It is relatively inactive biochemically. Acid but no gas is produced promptly from dextrose, rhamnose and salicin; the fermentation of sucrose, maltose, lactose, glycerol and starch is irregular and slow. Arabinose, galactose, xylose, mannitol, dulcitol, inulin and inosite are not

[41] Murray, Webb and Swann: Jour. Path. Bact., 1926, *29*:407.

[42] It has been pointed out that the name *Listerella* had been previously used for a Mycetozoan and for one of the Foraminifera, and the name *Listeria* was suggested by Pirie (Science, 1940, *92*:383) as a substitute conforming to the rules of nomenclature. The name *Listerella* and the term listerellosis appear to have become firmly established and will no doubt continue to be generally used.

fermented. Gelatin is not liquefied, indol is not produced, and nitrates are not reduced to nitrites.[43]

Julianelle and Pons[44] found that a large group of representative strains fell into two serologic types by agglutination and precipitin tests. One of these was associated with rodent infections and one with ruminant infections.

Pathogenicity for Animals.—Natural infection of animals has been observed with some frequency and appears to be of greatest importance in ruminants in which it is usually associated with encephalitis or encephalomyelitis. Encephalitis likewise commonly occurs in infected swine, while a generalized infection is usually found in rodents and fowls.[45] The mouse, guinea pig, rabbit and rhesus monkey may be infected experimentally, the mouse being the most susceptible. Strains of the bacterium vary considerably in virulence, the most virulent producing a fatal infection in mice and guinea pigs by intraperitoneal inoculation of 10^{-6} cc. of twenty-four-hour broth culture. In the rabbit a marked increase in circulating monocytes is often produced and at autopsy multiple necrotic foci in the liver, meningeal inflammation and myocardial abscesses are found. Julianelle has described a characteristic conjunctivitis and keratitis that may be produced in the eye of the rabbit by instillation or swabbing and which serves to differentiate this bacterium.

Pathogenicity for Man.[46]—This bacterium is of undoubted pathogenicity as indicated by reports of a number of cases of *Listerella* meningitis in which the case fatality rate has been about 70 per cent. Listerellosis in man does not appear to be common, however; about twenty cases have been reported in all. It is presumed that the infection is acquired from lower animals but there is no proof of this. Infection in man has been of particular interest in connection with the etiology of infectious mononucleosis.

Infectious Mononucleosis (Glandular Fever).—An acute, rarely fatal disease of man known as infectious mononucleosis or glandular fever is characterized by fever, sore throat, enlargement of the lymphatics and spleen, and a lymphocytosis. Heterophile antibody is found and the agglutination of sheep red cells, the Paul-Bunnell test, is diagnostic in the non-specific sense that it is associated with the disease.[47] *Listerella monocytogenes* has been isolated from a number of cases of the disease and this, coupled with the ability of the bacterium to produce a monocytic leucocytosis in experimental animals, has led a number of workers to suspect an etiological relationship. Too few cases have been studied to establish this; such evidence as there is has been critically reviewed by Webb.[48]

[43] For detailed studies see the review by Julianelle: Jour. Bact., 1941, *42*:367; and the studies of Harvey and Faber: Jour. Bact., 1941, *42*:677.

[44] Julianelle and Pons: Proc. Soc. Exp. Biol. Med., 1939, *40*:364.

[45] See the review by Graham, Levine and Morrill: Bull. Illinois Agr. Exp. Station. No. 499, 1943.

[46] *Cf.* Julianelle: Ann. Int. Med., 1940, *14*:608.

[47] For a survey of the literature on this disease see Smeall: Edinburgh Med. Jour., 1942, *49*:291.

[48] Webb: Lancet, 1943, *ii*:5.

NON-SPORE-FORMING ANAEROBIC BACILLI

There is a large group of non-spore-forming anaerobic bacilli that are usually gram-negative. Normal inhabitants of the upper respiratory tract, genital tract and colon, where they may outnumber the aerobic flora, the microorganisms are not infrequently associated with ulcerative processes of the mucous membranes and may, under appropriate circumstances, invade the tissues and organs of the body with the production of abscesses, or the blood stream to give rise to septicemia. Generally neglected in routine bacteriological examinations, these bacteria may be present in "sterile pus" from surgically drained abscesses and similar affections where bacteria are not found by the usual cultural methods. Dack[49] has noted their presence in 200 of 5180 specimens from the Department of Surgery of the University of Chicago submitted for routine bacteriological examination, an incidence of about 4 per cent.

The relation of these microorganisms to other bacteria is uncertain. As a group they probably make up several genera and cannot be regarded as species of a single genus except tentatively. They are morphologically heterogeneous, varying from slender rod forms which may be tapered toward the ends, the so-called fusiform bacilli, to filamentous and branching forms characteristic of the higher fungi. Single species have been given a variety of generic names, including *Bacillus*, *Bacterium*, *Necrobacillus*, *Bacteroides*, *Corynebacterium*, *Streptothrix* and *Actinomyces*. Descriptions of these bacteria have been compiled by Weinberg, Nativelle and Prevot,[50] and Prevot[51] has suggested a more or less elaborate classification. Bergey (1939) places these bacteria in two genera, *Fusobacterium* and *Bacteroides*, the latter little more than a listing of the forms described by Prevot. In the absence of further information neither the relation of these anaerobic forms to other bacteria nor their relation to one another can be satisfactorily formulated, and they will be discussed here under the single generic name *Bacterium*.

Those forms which are present in great numbers in normal feces will grow upon the usual laboratory media. Those which are associated with pathological processes in man, however, are nutritively fastidious and require infusion media enriched by the addition of blood, yeast or vegetable extracts and similar substances, together with glucose and cysteine. In some cases they may be isolated on beef or veal infusion blood agar. West, Lewis and Militzer[52] have been able to grow some strains in amino acid media supplemented with all the known bacterial vitamins; pyruvic acid appeared to be of considerable importance in the nutrition of these organisms. The optimum pH is 6.3 to 7.0 and the optimum temperature for growth is 37° C. Completely anaerobic conditions are essential and growth is favored by the presence of carbon dioxide.

The better known of these bacteria may be described briefly.

[49] Dack: Bact. Rev., 1940, *4*:227.

[50] Weinberg, Nativelle and Prevot: *Les Microbes Anaerobies*. Masson et Cie. Paris. 1937.

[51] Prevot: *Manuel de Classification et de Determination des Bacteries Anaerobies*. Monographies de l'Institut Pasteur. Masson et Cie. Paris. 1940.

[52] West, Lewis and Militzer: Jour. Bact., 1942, *43*:155.

Bacterium Fusiformis (*Bacillus fusiformis, Fusiformis fusiformis, Fusobacterium plauti-vincenti*).—These fusiform bacilli are found in ulcerative stomatitis (trench mouth) and Vincent's angina. Their relation to the spirochetes with which they are usually associated is considered elsewhere (p. 680). These forms are regarded by Bergey as the type species of the genus *Fusobacterium*. They appear as slender rectilinear or incurving bacilli and frequently assume a filamentous form. They are gram-negative and tend to stain irregularly with the ordinary aniline dyes. Acid but no gas is produced from dextrose, levulose, sucrose, maltose, and sometimes from lactose. They may be isolated on blood agar incubated anaerobically, and the colonies are small and surrounded by a zone of green hemolysis. *Bacterium fusiformis* is not pathogenic for experimental animals in pure culture, but in mixed culture produces abscesses.

Bacterium Fragilis (*Bacillus fragilis, Bacteroides fragilis, Fusiformis fragilis*).—This bacterium was found by Veillon and Zuber[53] in twenty-two cases of appendicitis and since has been found in lung, pelvic and hepatic abscesses, in septicemias with metastatic abscesses, and in infections of the urinary tract. The cells are small, slender rods, sometimes slightly curved. They are gram-negative and non-motile. *Bacterium fragilis* is difficult to isolate but will grow on the usual laboratory media. The colonies are small (less than 1 mm.) and transparent. No hemolysis is apparent on blood agar. There is a marked tendency to autolysis with apparent resorption of the colonies and broth cultures are no longer viable after seven to eight days' incubation. A variety of sugars are fermented to acid and gas. The pathogenicity of this bacterium for experimental animals is uncertain.

Bacterium Necrophorum, Bacterium Funduliformis (*Actinomyces necrophorus, Fusiformis necrophorus, Streptothrix necrophorus, Bacillus necrophorus, Corynebacterium necrophorum*, Schmorl's bacillus).—According to Dack[49] *Bacterium necrophorum* and *Bacterium funduliformis* are not separate species. These bacilli have been found in abscesses of the liver, lung and other parts of the body, in chronic ulcerative colitis and in the blood stream. Infections with these microorganisms are probably more common than is generally realized.[54] *Bact. necrophorum* has also been found in lower animals as in bovine liver abscesses. The bacilli are highly pleomorphic; slender straight and curved rods may be found intermingled with filamentous and swollen forms, and "ghost cells" which do not stain are frequent (Fig. 110). There is a marked tendency to irregular staining and the bacilli are gram-negative. The colonies on blood agar are variable in size from plate to plate and surrounded by a zone of green hemolysis which may become clear upon prolonged exposure to the air. Glucose, maltose and levulose are fermented to acid, and there is no evidence of proteolytic activity in gelatin or coagulated egg-white cultures. Some strains give rise to a spreading necrotic lesion upon subcutaneous injection in the rabbit which is usually fatal, while others produce only a localized lesion. Guinea pigs are relatively resistant.

[53] Veillon and Zuber: Arch. de Med. Exp., 1898, *10*:517.

[54] *Cf.* Lemierre: Lancet, 1936, *i*:701; Brunner: Münch. med. Wchnschr., 1937, *84*: 2032.

Bacterium Ramosum (*Bacteroides ramosus, Fusiformis ramosus, Ramibacterium ramosum, Bacillus ramosus.* Not to be confused with *Bacillus ramosus*, a name sometimes applied to *Bacillus mycoides*).—These bacteria are frequently present in the pus of appendicitis and have been encountered in pulmonary gangrene.[55] They appear as small, slender rods which often show branching Y forms and pseudofilaments. They are gram-positive. *Bact. ramosum* is pathogenic for experimental animals, giving rise to abscesses in rabbits and guinea pigs on subcutaneous injection; fatal infections are produced by intravenous inoculation.

Bacterium Melaninogenicum (*Bacteroides melaninogenicus, Ristella melaninogenica*).—This microorganism has been found in the mouth, tonsils, infected abdominal wounds, focal infections of the kidneys, in stools from patients with chronic amebic dysentery, and in puerperal sepsis. It is described by Burdon[56] as a very small gram-negative diplobacillus. On

Fig. 110.—*Bacterium necrophorum.* A typical preparation showing the pleomorphism of this bacterium. Note the swollen and filamentous forms and the poorly staining "ghost cells" (Dack).

blood agar its colonies are black owing to the slow (four to five days) formation of a melanin-like pigment. *Bact. melaninogenicum* grows well in mixed culture, but sparsely in pure culture. It is difficult to obtain in pure culture and, when admixed with other bacteria in a colony, colors the entire colony black. Acid is formed from dextrose, levulose, lactose, maltose, sucrose and mannitol. It is markedly proteolytic and rapidly digests coagulated serum and other native proteins. Its pathogenicity as a primary invader is uncertain.

Bacterium Pneumosintes (*Dialister pneumosintes*).—*Bacterium pneumosintes* was described by Olitsky and Gates[57] as the causal agent of influenza. Now known to bear no relation to this disease, its pathogenicity for man is uncertain. It may be cultured from nasopharyngeal washings in

[55] *Cf.* Jour. Amer. Med. Assn., 1937, *108:*1902.

[56] Burdon: Jour. Inf. Dis., 1928, *42:*161.

[57] Olitsky and Gates: Jour. Exp. Med., 1921, *33:*125, 361, 713; *ibid.*, 1922, *35:*813; *ibid.*, 1922, *36:*501.

Smith-Noguchi medium (human ascitic fluid containing a piece of sterile rabbit kidney, and covered with a vaseline seal) and after a few transfers will grow anaerobically on blood agar, chocolate agar and Bordet's medium. These bacteria are minute bodies arranged singly, in pairs, or in short chains; they are non-motile and gram-negative. Because of their small size they pass Berkefeld V and N filters. They produce acid but no gas from dextrose; other sugars are not fermented. If mass cultures are injected intratracheally into rabbits there is a rise in temperature and sometimes a conjunctivitis and mononuclear leucopenia with recovery in two to three days. If the animal is sacrificed the lungs are found to be edematous with hemorrhages on the surfaces. *Bact. pneumosintes* is not pathogenic for monkeys.

BARTONELLA

Bartonella Bacilliformis.—Oroya fever, an infectious anemia, and verruga peruana, a disease characterized by miliary or nodular eruptions, have existed for centuries in certain districts in Peru, and recently have

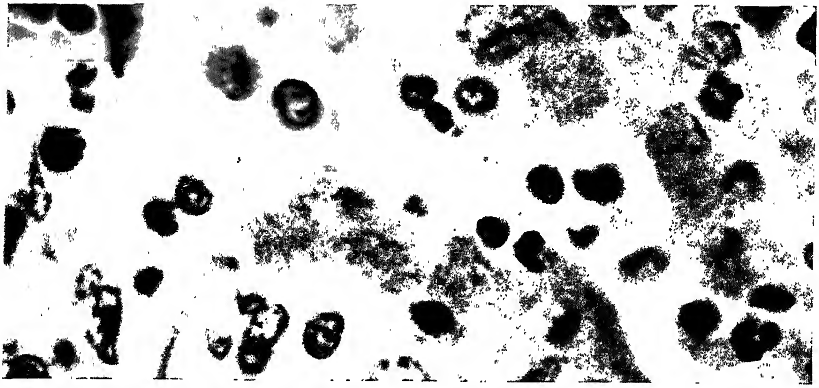


Fig. 111.—*Bartonella bacilliformis* in human spleen. Note the huge numbers of the microorganisms packed into the lining cells. Giemsa; × 1450 (Humphreys).

been found in Colombia and Ecuador. It was shown by Carrion, through fatal self-inoculation, that the two are stages or manifestations of a single disease which is now commonly known as *Carrion's Disease.* The etiologic agent is a small pleomorphic bacillus which was observed by Barton in 1905 and named *Bartonella bacilliformis* by Strong, Tyzzer and Sellards.[58]

Morphology and Staining.—Bartonella bacilliformis is a small, motile, aerobic, gram-negative bacillus 0.2 to 0.5 μ in diameter and 1 to 2 μ in length which is found as a slightly curved rod occurring singly, end to end in pairs, and in short chains. A rounded ovoid form, 0.3 to 1 μ in diameter, is also observed singly, in pairs and in groups. It stains reddish violet with Giemsa's stain, sometimes showing a reddish purple granule at one end of a bluish rod.

[58] Strong, Tyzzer and Sellards: Jour. Amer. Med. Assn., 1915, *64*:806.

Physiology.—This microorganism was first cultivated in semi-solid leptospira medium[59] and later in tissue culture[60] and in the developing chick embryo,[61] though it could not be carried in serial transfer in the last. Sparse growth occurs on cystine-dextrose-blood agar and apparently the X factor is required while the V factor is not.[62] Gieman[63] has developed a tryptone-serum medium in both liquid and solid form which supports excellent growth. Since it has been cultivated with reasonable facility only recently little is known of its physiological processes.

Pathogenicity for Man.—As indicated above, the disease occurs in two forms, the systemic form in which the red cells are infected, and the histoid cutaneous form. The two may exist independently, coexist or occur separately, though the usual course is the systemic form followed by the cutaneous form if the former is not fatal, or the cutaneous form alone. The first is a severe, often fatal, febrile anemia. The incubation period is given as about three weeks. The anemia is frequently severe, and the loss of red cells may be 200,000 to 300,000 per cubic millimeter per day until the total count is half a million or less. The anemia is thought to be due to the direct action of the microorganisms on the erythrocytes, including hemolysis, and to tissue hemorrhages. The fever is of an irregular remittent type, and pain in the bones, joints and head is common. The case fatality rate is 20 to 40 per cent, death occurring in two to three weeks after onset. With recovery the eruptive stage of the disease appears and persists for two to three months. Whether it follows the systemic form or is the primary clinical manifestation of infection, this stage is characterized by a miliary or nodular eruption; the former is by far the more common. The miliary eruption is most common on the face and extremities, appearing as a macule which becomes nodular and eventually disappears leaving no scar. The nodular type of eruption develops more slowly, the nodules may become 2 to 3 cm. in diameter and have a tendency to become strangulated. They are formed by the proliferation of the endothelial cells of the vessels which become obstructed with an inflammatory exudate of plasma cells and fibroblasts, and show a marked tendency to hemorrhage.

Bartonella cells are found in large numbers within the erythrocytes in Oroya fever and may be demonstrated in Giemsa-stained blood smears. In both forms of the disease they are found in the tissue macrophages, especially the vascular endothelial cells of the lymphatics, spleen and liver, often in large clusters within individual cells. It is reported that blood cultures are frequently positive in the systemic infection but it is not clear whether this is a reliable method of diagnosis.

Epidemiology.—Bartonellosis is strictly limited geographically, being, so far as is known, exclusively American and tropical, occuring in the Andes between latitudes 2° N. and 13° S. It is transmitted by *Phlebotomus verrucarum* and *P. noguchii* in Peru; whether other species of *Phlebotomus* or

[59] Noguchi and Battistini: Jour. Exp. Med., 1926, *43*:851.
[60] Pinkerton and Weinman: Proc. Soc. Exp. Biol. Med., 1937, *37*:587.
[61] Jiminez and Buddingh: Proc. Soc. Exp. Biol. Med., 1940, *45*:546.
[62] Jiminez: Proc. Soc. Exp. Biol. Med., 1940, *45*:402.
[63] Gieman: Proc. Soc. Exp. Biol. Med., 1941, *47*:329.

other arthropods, such as *Dermacentor*, are natural vectors is not known. The reservoir of infection, other than asymptomatic infections and clinical disease in man, is not known but domestic animals, including chickens, and guinea pigs and field mice are suspected.[64]

Immunity.—It is generally said that recovery from an attack of either form of the disease confers a solid immunity to both. With the cultivation of *B. bacilliformis*, it has been possible to study the occurrence of circulating antibody, agglutinins, and investigate the possibilities of prophylactic inoculation. Howe[65] has shown that agglutinins may be demonstrated during the early stages of the disease but, in spite of the lasting immunity, almost always disappear with the subsidence of clinical symptoms. Their diagnostic significance is not as yet known. Howe and Hertig[66] have carried out a limited series of inoculations with formolized suspensions of *B. bacilliformis* with encouraging results.

Pathogenicity for Animals.—In general experimental animals appear to be highly resistant to infection with *B. bacilliformis*. Both the eruptive and systemic forms of the disease have been produced in rhesus monkeys, the latter in splenectomized animals.

Bartonellosis of Animals.—Naturally occurring bartonellosis has been observed in a number of animals; this takes the form of systemic infection rather than a cutaneous or eruptive one. In many instances the infection is latent, the host having attained an equilibrium with the parasite. If the host resistance is reduced by splenectomy the infection becomes acute.

Bartonella muris.—Bartonellosis of rats was demonstrated by Mayer[67] and the infection is very common. It is almost always latent and is precipitated in an acute form by splenectomy with the appearance of parasitized red cells. It is transmitted from rat to rat by the rat louse *Hematopinus* and the rat flea *Xenopsylla cheopsis*. The presence of the latent infection is of some importance in rat experiments but certain strains are maintained bartonella-free.

Bartonella canis.—This microorganism was found by Kikuth[68] to be the causative agent of an infectious anemia of dogs. It is considerably more pleomorphic than *B. bacilliformis* and is transmitted by the dog flea, *Ctenocephalus*.

Bartonella tyzzeri.—This microorganism was isolated from guinea pigs by Weinman and Pinkerton.[69] It has been found in wild guinea pigs in South America and occurs as a latent infection.

Bartonella microti.—This microorganism is a parasite of the field vole and is morphologically similar to *B. canis*. Its vector is not known.[70]

The Systematic Position of Bartonella.—The relationship of these microorganisms to the bacteria on the one hand and to the rickettsiae and

[64] For an excellent review of the present status of human bartonellosis see Mera: Bol. Oficina Sanitaria Panamericana, 1943, *22*:304.

[65] Howe: Jour. Exp. Med., 1942, *75*:65; Arch. Int. Med., 1943, *72*:147.

[66] Howe and Hertig: Jour. Immunol., 1943, *47*:471.

[67] Mayer: Arch. f. Sch. u. Trop. Hyg., 1921, *25*:150.

[68] Kikuth: Centralbl. f. Bakt., I Abt., Orig., 1929, *113*:1.

[69] Weinman and Pinkerton: Ann. Trop. Med. Parasitol., 1938, *32*:215.

[70] Tyzzer and Weinman: Amer. Jour. Hyg., Sec. B., 1939, *30*:141.

viruses on the other is of some interest. Among the bacteria the tendency to parasitize the cells of the host and appear as intracellular clumps of microorganisms is most marked in *Pasteurella tularensis* which commonly is found in an intracellular position. *Bartonella* shows a much more marked preference for intracellular parasitism and both in this respect and in morphology seems to be closely related to the rickettsiae. It is generally agreed, however, that the relationship is not sufficiently close to justify their classification as rickettsiae and they may be considered as lying between them and the bacteria.

THE PLEUROPNEUMONIA-LIKE ORGANISMS[71]

The first of an apparently more or less homogeneous group of highly pleomorphic, filterable microorganisms to be described is the causative agent of *bovine pleuropneumonia.* Since its first study in 1898[72] a number of similar forms, both parasitic and saprophytic, have been found. For want of a better name they have been called the pleuropneumonia group or pleuropneumonia-like organisms, though none of the rest produces pleuropneumonia in cattle. Their relationship to other microorganisms is almost completely unknown and they have been grouped with the viruses by some because of their filterability, with the rickettsiae by others because of a predilection for an intracellular existence in the host, and, as indicated earlier, by still others they are grouped with the actinomycetes.

Morphology and Staining.—There are probably few if any other microorganisms which are as highly pleomorphic as these forms. Impression preparations of colonies show granules of various sizes, filaments which may be branched and contain streaming protoplasm, balloon and disk-like structures, ring, club and star forms, and ameboid structures. Filaments are more numerous in recently isolated cultures and tend not to be formed in older stock cultures. It has been urged by some[73] that these structures represent stages in a cyclical developmental process, but this interpretation has not been generally accepted. Viable structures vary greatly in size and include ultramicroscopic elements which are filterable. Edward[74] describes experiments on filtration through gradocol membranes (p. 788) in which the concentration of organisms in an emulsion was reduced from 10^8 to 10^5 by passage through a membrane of APD 0.8 μ. This titer decreased progressively with membranes of decreasing pore size but complete retention did not occur until a membrane of 0.33 μ was used. In contrast to this, the end-point is usually sharp in the filtration of viruses. On the basis of these observations the diameter of the smallest viable elements was estimated to be 165 to 247 μ. Similar estimates vary somewhat for other strains; the agent of pleuropneumonia, for example, is 125 to 175 μ.

These forms cannot be found in tissues by any method of staining. They

[71] These microorganisms are discussed in detail in the review by Sabin: Bact. Rev., 1941, *5*:1.

[72] Nocard and Roux: Ann. Inst. Pasteur, 1898, *12*:240. See also Dujardin-Beaumetz· Thesis. Gaston & Cie. Doin. Paris. 1900.

[73] Tang, Wei, McWhirter and Edgar: Jour. Path. Bact., 1935, *40*:391.

[74] Edward: Jour. Path. Bact., 1940, *50*:409.

are not demonstrable in smears stained by the usual aniline dyes, but are stained by certain polychrome stains, Giemsa and Castaneda's rickettsia stain. Very thick films prepared from the sediment of centrifuged cultures are gram-negative.

On primary isolation or change to a slightly different culture medium, broth cultures sometimes, but not always, show no detectable evidence of growth and may be carried along by "blind passage" for several transfers before it appears. Some strains show a uniform opalescence, others a granular type of growth. The characteristic of some strains to grow as small colonies, appearing as flakes attached to the side of the tube, is of some differential value. In any case, visible evidence of growth is very slight and almost all workers with these organisms have found it necessary to carry along an uninoculated tube of medium for comparative purposes.

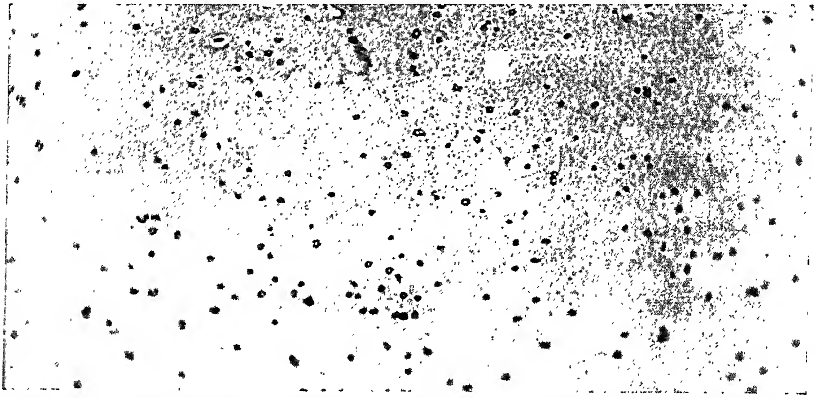


Fig. 112.—Two-day culture of Type A pleuropneumonia-like organism from mice in serum-dextrose broth. Note the rings and elementary bodies. Giemsa; × 1000 (Sabin, Bact. Rev.).

The colonies upon an agar surface are usually not detectable until after two or three days' incubation and then ordinarily only with a hand lens. The average colony size of different strains ranges from 0.1 to 0.6 mm., rarely exceeding the larger figure. The colonies usually have a dark center with a lighter margin, and may appear coarsely or finely granular. Upon higher magnification a foam-like structure may be observed which is composed of the balloon-like forms. Oily droplets may be found in the colonies of some strains; these have been found, in some cases at least, to be largely cholesterol already present in the medium.[75] Possibly the pseudo colonies which have been observed[76] on uninoculated serum agar are of similar nature; it is said that these are readily differentiated from true colonies by experienced observers.

Physiology.—These microorganisms require infusion or digest media enriched by the addition of serum or ascitic fluid in relatively large amounts.

[75] Partridge and Klieneberger: Jour. Path. Bact., 1941, *52*:219.
[76] Brown, Swift and Watson: Jour. Bact., 1940, *40*:857.

Various workers add from 10 to as high as 40 per cent serum; 50 per cent and over is inhibitory for some strains. Some have included boiled blood and fresh serum, and small amounts of glucose facilitate primary isolation of some strains. In some cases these organisms tolerate a relatively wide *p*H range, but others die out at *p*H 7.0 and below and require a *p*H of 7.8 to 8.0 for growth. The saprophytic varieties will grow at 22° C. with an optimum at 30° C. but the parasitic ones require 37° C. Growth occurs both aerobically and anaerobically, but is less abundant with most strains under anaerobic conditions. Fairly heavy inocula are required, 0.1 to 0.2 cc. of minced tissue in primary isolation, the transfer of similar amounts of broth cultures, and in transfer from agar cultures a small section of the medium is cut out and dropped into liquid media or streaked on another plate. These organisms may also be cultured on the chorioallantoic membrane

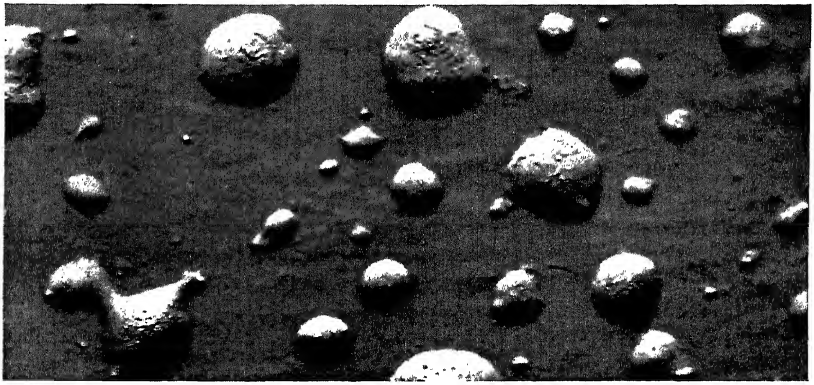


Fig. 113.—Colonies of a Type B pleuropneumonia-like organism from mice. Three days' incubation. × 100. (Sabin, Bact. Rev.)

of the developing hen's egg.[77] Some sugars, notably glucose, are fermented but the *p*H seldom drops below 7.0 owing to the death of the organisms at this point; the addition of other sugars may inhibit growth. In general, fermentations and other biochemical characteristics have no differential value.[78] The heat resistance of some strains is of the same order as that of most bacteria, while others appear to be more frail and are killed by exposure to 45° C. for fifteen minutes. Cultures are best preserved at incubator temperature in media containing no added sugar; when sealed with petrolatum the organisms may remain viable for a month or more.

Varieties, Strains or Species.—Strains of these organisms have been isolated from a variety of sources. Those which are parasitic are definitely set off from the saprophytic types, and differentiation or identification within these groups has been made in part on source and pathogenicity, and in part on an immunological (agglutination) basis.

[77] Swift: Jour. Exp. Med., 1941, *74*:557.
[78] *Cf.* Warren: Jour. Bact., 1942, *43*:211.

Bovine Pleuropneumonia.—As indicated above, the causative organism of pleuropneumonia of cattle was the first of these forms to be isolated and studied. The disease is found all over the world except in India, Western Europe and North America; it has been imported into this country a number of times but was finally eradicated by slaughter of infected animals and has not occurred since 1892.

The natural disease in cattle is characterized by extensive consolidation and subpleural effusion in either or both lungs and the microorganism is present in large numbers in the serous exudate. It spreads slowly in herds and may take an acute form with death within a week or a chronic form with walling off of the foci of infection. There is occasional joint involvement in young animals. The natural disease has not been reproduced in cattle by either inoculation of infectious serous exudate or of cultures, but an extensive edema develops and spreads from the site of inoculation, and there is a febrile reaction and sometimes death.

The organism is apparently completely non-pathogenic for the usual experimental animals and for man; it has been reported[79] that cultures in sheep- or horse-serum media are infectious for sheep and goats, while those in bovine-serum media are not.

The name *Asterococcus mycoides* was given the organism by Borrel and Dujardin-Beaumetz[80] but has not been generally used. Sabin[71] has proposed the name *Bovimyces pleuropneumoniae.* In any case, strains obtained in different localities and at different times appear to be very similar if not identical.

Contagious Agalactia of Sheep and Goats.—Despite its name this disease is a generalized infection which affects both males and females. It occurs only in parts of southern Europe and North Africa; agalactia or mastitis of these animals in this country is of other etiology.

The lesions occur in the joints and eyes and in the mammary glands of females. The microorganism is present in the blood early in the disease and later may be isolated from the affected regions and from the mammary secretions. The second member of the group to be studied, it was isolated by Bridré and Donatien[81] in 1923. It is morphologically and biochemically very similar to the pleuropneumonia organism and other members of the group, but is immunologically distinct and of characteristic pathogenicity. Sabin[71] has suggested that it be called *Capromyces agalactiae.* The disease is readily reproduced by inoculation of sheep and goats with cultures.

Canine Variety.—Organisms of the pleuropneumonia group have also been found in dogs. Shoetensack[82] reported in 1934 the cultivation of an organism of this type from the purulent nasal discharge of dogs ill with distemper which he called *Asterococcus canis.* In later studies other organisms were found and there appeared to be two types, immunologically distinct from one another, which he called Type I and Type II respectively. Sabin[74] proposed that these be called *Canomyces pulmonis I and Canomyces pul-*

[79] Dujardin-Beaumetz: Ann. Inst. Pasteur, 1906, *20*:449.
[80] Borrel and Dujardin-Beaumetz: Ann. Inst. Pasteur, 1910, *24*:168.
[81] Bridré and Donatien: C. R. Acad. Sci., 1923, *177*:841.
[82] Shoetensack: Kitasato Arch. Exp. Med., 1934, *11*:277.

monis II. Their postulated[83] etiological relationship to distemper, generally regarded as a virus disease, is not established.

Varieties from Rats.—A series of pleuropneumonia-like organisms has been isolated by Klieneberger and her associates[84] from the respiratory tract and elsewhere in normal and diseased rats. Others[85] have isolated similar organisms from rats exhibiting polyarthritis and swollen extremities. These comprise, with a single exception, the "L" series of strains in which the strains are designated by subscript numerals. There now appear to be three distinct types, differentiated on a biological and immunological basis, *viz.*, L_1, L_3, and L_4. The strain L_2 was isolated from a guinea pig and insufficiently studied before loss of the culture at the outbreak of war. The strain described as L_5 is immunologically identical with mouse type A discussed below; L_6 has been insufficiently studied; and L_7 has been found to be identical with L_4.

Considerable interest has attached to the apparent association of L_1 with *Actinomyces muris-ratti*,[86] Klieneberger has been able to isolate L_1 from many strains and stock cultures of *A. muris-ratti* and it will be recalled that this fungus is a natural parasite of the nasopharynx of rats and mice. It is maintained by Klieneberger[87] that L_1 and the fungus coexist as symbionts since she has been able to separate the two on the basis of differential resistance to heat and aging, has observed L_1 in the rat in the absence of *A. muris-ratti*, and has carried L_1 strains through many transplants without reappearance of the actinomycete. Dienes,[88] however, has taken the view that L_1 and *A. muris-ratti* represent but stages in a hypothetical cyclogeny. This has not been generally accepted but it is not as yet apparent whether the virulence of either organism is affected by the presence of the other.

These L forms are associated, causally in some instances at least, with relatively mild, chronic affections of rats in which a not uncommon manifestation is joint involvement and polyarthritis. They closely resemble the other organisms of the group morphologically and culturally, forming a subgroup on the basis of pathogenicity, and differentiated from one another immunologically. Sabin[71] has grouped L_1, L_3, and L_4 under a single genus and suggested the names *Murimyces streptobaccilli-moniliformis*, *Murimyces pulmonis*, and *Murimyces arthritidis* respectively for them.

Varieties from Mice.—Sabin[71] divides the pleuropneumonia-like organisms found in mice into five types, designated as A, B, C, D and E. Type A is found in normal mice, once in the brain and frequently in the eyes, nasal mucosa and lungs of carriers. On intracerebral inoculation of mice an ataxia

[83] Shoetensack: Kitasato Arch. Exp. Med., 1936, *13*:175, 269.

[84] Klieneberger: Jour. Path. Bact., 1935, *40*:93; *ibid.*, 1936, *42*:587; Klieneberger and Steabben: Jour. Hyg., 1937, *37*:143; Klieneberger: Jour. Hyg., 1940, *40*:204.

[85] Woglom and Warren: Jour. Exp. Med., 1938, *68*:513; Sabin: Science, 1938, *88*:575; *ibid.*, 1939, *90*:18: Findlay, Klieneberger, MacCallum and Mackenzie: Lancet, 1939, *i*:7.

[86] It may be noted that the name *Streptobacillus moniliformis* continues to be used exclusively in the pleuropneumonia group literature in spite of its impropriety and almost always with no mention of its synonyms. The unnecessary confusion introduced is unfortunate. See page 614.

[87] In several papers; *Cf.* Klieneberger: Jour. Hyg., 1942, *42*:485.

[88] Dienes: Jour. Inf. Dis., 1939, *65*:24. Also Heilman: *ibid.*, 1941, *69*:32, 45.

is produced which is characterized by a turning or rolling of the body. The brain lesions and symptoms arising from them are attributable to the action of a soluble toxin produced by the microorganisms.[89] An immunologically identical organism was isolated by Findlay *et al.* from mice affected with "rolling disease" and designated L_5. Type B[90] has likewise been found in normal mice and is not only immunologically distinct but produces a progressive arthritis almost exclusively when parenterally inoculated into mice. Types C, D and E are similar in their pathogenicity but are immunologically distinct; they have not been studied as extensively as some of the others. These murine forms are, like the L forms, grouped in a single genus by Sabin,[71] type A being given the name *Musculomyces neurolyticus*, type B the name *Musculomyces arthrotropicus*, and types C, D, and E grouped in the single species *Musculomyces histotropicus*.

Pleuropneumonia-like Organisms of Man.—Though there is considerable evidence suggesting that certain streptococci are associated with some types of arthritis in man (p. 326), the etiology of human arthritis is far from clear. The pleuropneumonia-like organisms have assumed some interest in this connection, largely because of the frequency of occurrence of progressive polyarthritis in both naturally and artificially infected rats and mice. The association of L_1 strains with *A. muris-ratti* is of possible interest in view of the arthritic manifestations of epidemic disease of the Haverhill fever type and of sporadic rat-bite fever of fungus etiology (p. 614).

In any case, a considerable number of attempts have been made to isolate microorganisms of this type in rheumatic and arthritic conditions of man. These have, without exception, failed. In one instance, however, such an organism was isolated from the vagina;[91] it has been found[92] to be non-pathogenic for experimental animals and immunologically different from L_3 and L_4 and A, B, C, D, and E mouse types. Also Beveridge[93] has reported the isolation of pleuropneumonia-like organisms from four of twenty-four males with non-gonorrheal urethritis. The significance of these organisms in human disease is as yet completely unknown.

Saprophytic Varieties.—Pleuropneumonia-like organisms presumably living a saprophytic existence in nature have been found by Laidlaw and Elford[94] in raw London sewage. They closely resembled the parasitic forms both morphologically and culturally, and fell into three immunological groups which were designated types A, B, and C. All were non-pathogenic for experimental animals. Similar forms have been found in Germany[95] in compost and other types of decomposing organic matter. Sabin[71] has proposed that these saprophytic forms be named *Sapromyces laidlawi*.

[89] Sabin: Science, 1938, *88*:189, 575.
[90] Sabin: Science, 1939, *89*:228. Also Jour. Bact., 1940, *39*:343.
[91] Dienes and Smith: Proc. Soc. Exp. Biol. Med., 1942, *50*:99.
[92] Warren and Sabin: Proc. Soc. Exp. Biol. Med., 1942, *51*:24.
[93] Beveridge: Med. Jour. Australia, 1943, *30*:479.
[94] Laidlaw and Elford: Proc. Roy. Soc. (London) Ser. B, 1936, *120*:292.
[95] Seiffert: Centralbl. f. Bakt., I Abt. Orig., 1937, *139*:337; *ibid.*, 1937, *140*:168.

CHAPTER 27

THE ANTHRAX BACILLUS

Family: *Bacillaceae.* Genus: *Bacillus.* Aerobic, spore-forming rods. Usually gram-positive. Most species saprophytes found in the air, soil and dust. Type species: *Bacillus subtilis.*

The spore-forming rod-shaped bacteria are divided into two groups on the basis of their relation to atmospheric oxygen. The *Bacilli* are the aerobic forms and the anaerobic types are designated *Clostridium* (Chap. 28). A very large number of species of *Bacilli* have been described, the majority of them from soil and dust. Two species, *Bacillus alvei* and *Bacillus paraalvei,* cause foulbrood, a disease of bees. *Bacillus subtilis* infects human beings only rarely, and, with this exception, *Bacillus anthracis* is the only member of this large group that is pathogenic for man.

Primarily a disease of lower animals transmissible to man, anthrax (splenic fever; Fr. *charbon;* Ger. *Milzbrand*) is of particular historical interest, for it was in his study of this disease that Koch provided the first demonstration of the causal relation between a specific bacterium and an infectious disease. The bacillus had been observed in the blood and organs of animals dying of anthrax by Davaine and Rayer[1] in 1850 and by Pollender[2] in 1855. Brauell[3] transmitted the disease by the inoculation of blood from infected animals in 1857. Conclusive demonstration of the causal relation between the bacilli and the disease, however, was the work of Koch,[4] who in 1877 cultured the bacillus on the aqueous humor of the ox's eye, described its life history, and reproduced the disease with a pure culture of the microorganism. The importance of this discovery to the development of bacteriology has been discussed elsewhere (Chap. 1).

Morphology and Staining.—The anthrax bacillus is one of the largest of the pathogenic bacteria and ranges from 4.5 to 10 μ in length and from 1 to 1.25 μ in breadth. The ends of the rods are often concave and somewhat swollen so that the appearance of a chain of anthrax bacilli has often been compared to a jointed bamboo fishing rod. The cells occur singly and as end-to-end pairs or short chains in the body, but in culture long chains are formed. Unlike most of the sporulating aerobic bacilli they are non-motile.

Capsules may be found on the bacilli in smears from an infected animal but are not found in culture except on media rich in animal protein, such as serum agar. The capsular material is not polysaccharide as it is in most bacteria, but is a high molecular weight polypeptide composed exclusively

[1] Davaine and Rayer: Bull. Soc. de Biol., 1850, p. 141.
[2] Pollender: Vierteljahr. f. ger. Med., 1855, *8*:103.
[3] Brauell: Arch. f. path. Anat., 1857, *11*:132.
[4] Koch: Cohn's Beiträge, 1877, 2:277.

of d(-)glutamic acid (the "abnormal" stereoisomer).[5] This is a point of particular interest, for it is the first demonstrated natural occurrence of d(-)glutamic acid and of a polypeptide composed of a single amino acid. There is, in addition, a polysaccharide haptene present in the cell substance of the bacilli, which may be isolated from these bacilli; it appears to be the same in both virulent and avirulent strains and its immunological function is not clear.[6]

The anthrax bacillus also differs from most other pathogenic bacteria in that it forms spores which are visible as refractile bodies either free or located centrally within the cell. Their diameter does not exceed that of the vegetative cell and hence the spore-containing rod is not distorted. Spores are formed most abundantly at 32° to 35° C. and only under aerobic conditions, *i.e.*, not in the circulating blood of infected animals. Germina-

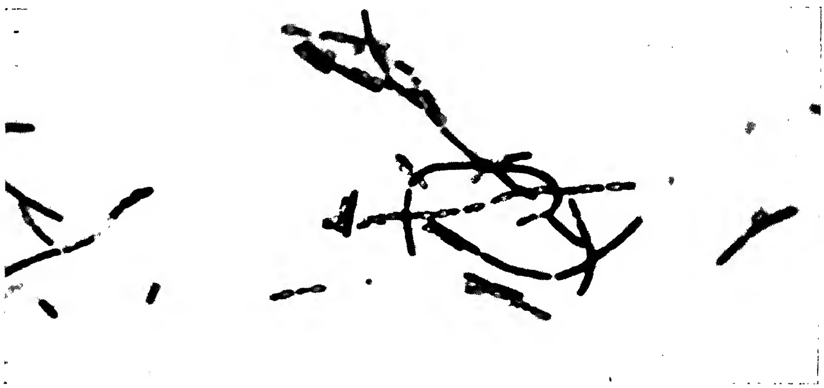


Fig. 114.—*Bacillus anthracis*, forty-eight-hour culture on nutrient agar. Crystal violet stain. The spores appear as unstained areas. Note the typical arrangement of the bacilli in coiled chains. × 1200.

tion of the spore is usually polar, that is, parallel with the long axis, but may be rarely equatorial.

The bacilli stain readily, but often unevenly, with the usual aniline dyes. The granular material within the cell consists of fat, volutin or glycogen. They are gram-positive. Churchman[7] has observed a reversal of the Gram reaction when aqueous gentian violet, acriflavin or acriviolet is added to suspensions of young cultures of *Bacillus anthracis*; a considerable proportion of the bacilli then become gram-negative. It has been concluded that the bacillus is made up of a gram-positive outer layer or cortex and a gram-negative medulla. When the former is destroyed the latter comes into view and the gram-negative bacilli are considerably smaller than the gram-

[5] Bruckner and Ivanovics: Ztschr. f. physiol. Chem., 1937, *247*:281; Ivanovics and Bruckner: Ztschr. f. Immunitätsf., 1937, *90*:304; *ibid.*, 1937, *91*:175; Naturwissenschaften, 1937, *25*:250.

[6] Ivanovics: Ztschr. f. Immunitätsf., 1940, *97*:443.

[7] Churchman: Jour. Exp. Med., 1927, *46*:1007.

positive cells. The spores are stained with difficulty, and after staining with hot carbol fuchsin are equally difficult to decolorize; hence the vegetative cells may be decolorized and stained with a contrasting dye.

The colonies of the anthrax bacillus are irregular and have a curled or hair-like structure, giving what is sometimes called a "Medusa head" appearance. On microscopic examination tangled coils of long chains of bacilli may be found. This colonial appearance is closely simulated by *Bacillus subtilis* and some other related saprophytic, aerobic, spore-forming bacilli.

Physiology.—The anthrax bacillus grows readily upon all the ordinary laboratory media, and growth is not improved by the addition of enriching substances. It will grow on simple amino acid media containing only glycine and cystine and no added bacterial vitamins.[8] Growth occurs at tempera-

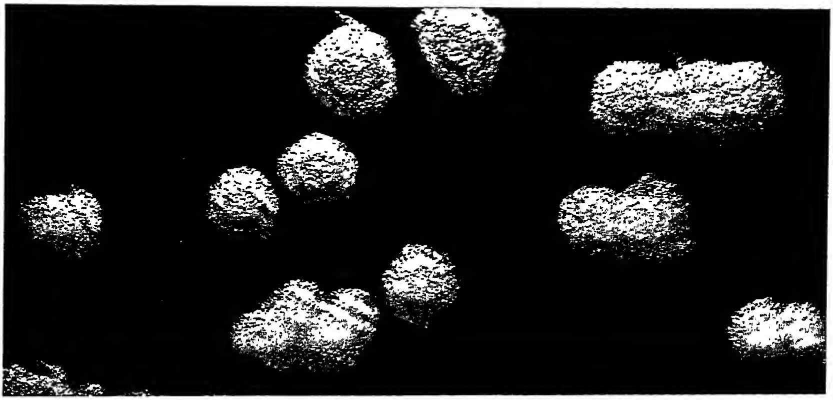


Fig. 115.—Colonies of *Bacillus anthracis* on nutrient agar. Twenty-four-hour culture. Note the large size and coarse texture suggestive of R variants. × 3.

tures as high as 41° to 43° C., with an optimum at 37° C. These bacilli are aerobic and facultatively anaerobic. Dextrose and trehalose are fermented rapidly but without gas production. Sucrose, maltose and some other carbohydrates are fermented less rapidly; lactose, galactose, mannitol, dulcitol, rhamnose and xylose not at all. Gelatin is slowly liquefied but indol is not formed, nitrate is not reduced, and little or no hydrogen sulfide is produced. Milk is feebly acidified and is curdled by a rennet-like ferment, and the casein slowly peptonized. On potato a gray, furry growth is produced; spores are formed in particular abundance on this medium.

No cultural or biochemical characteristics serve to differentiate the anthrax bacillus from the very similar nonpathogenic saprophytic sporulating bacilli; animal inoculation to determine pathogenicity is essential.[9]

The vegetative cells of the anthrax bacillus display the usual degree of resistance to deleterious influences, but the spores are relatively highly

[8] Gladstone: Brit. Jour. Exp. Path., 1939, *20*:189.
[9] See, for example, Stein: Amer. Jour. Vet. Res., 1944, *5*:38.

resistant, although not so resistant as the spores of *Bacillus subtilis* and related forms. Graham-Smith[10] has found that spores exposed to daylight at room temperature would germinate after twenty-two years but not after twenty-two and one-half years; spores dried on canvas and kept in envelopes would not germinate after thirty-five years. Anthrax spores are usually destroyed by boiling for ten minutes and by dry heat at 140° C. for three hours. Their resistance to disinfectants is variable, for 0.1 per cent mercuric chloride may fail to kill them in seventy hours, while those disinfectants which are oxidizing agents are much more effective; 3 per cent hydrogen peroxide kills in one hour and 4 per cent potassium permanganate in fifteen minutes. In the soil anthrax spores may remain viable for many years; some multiplication occurs in sterile soil but not in unsterilized soil.[11]

Variation.—The rough variant is the virulent and naturally occurring form of *B. anthracis*. It was early noted by Pasteur that prolonged cultivation of these bacilli at higher than optimum temperatures, 42.5° C., resulted in a loss of virulence and the appearance of asporogenous variants. A number of different types of variants are produced by such cultivation at high temperatures or in the presence of dilute antiseptics. Smooth and mucoid types of colonies have been observed, and some of these variants may be non-spore-forming. Virulence, however, is associated with the presence of a capsule, or the ability to form one, rather than the ability to form spores, for both asporogenous virulent strains and spore-forming avirulent strains may be found.[12] These probably do not occur in nature, however.

Pathogenicity for Lower Animals.—In nature anthrax is primarily a disease of cattle and sheep; horses and swine are susceptible, but are less commonly affected. Wild deer and other gregarious herbivora are liable to occasional outbreaks. The smaller rodents are very sensitive to inoculation. Rabbits, guinea pigs and white mice are susceptible in that order, and are fatally affected by the subcutaneous introduction of a very small number of virulent bacilli. The white mouse may succumb to inoculation with a single bacillus of a highly virulent strain. Carnivorous animals, though possessing greater resistance than the herbivora, are nevertheless susceptible, as several epidemics in zoological gardens involving leopards, lions, pumas, bears and other animals have shown. Certain animals possess a marked natural resistance to anthrax. Rats are quite resistant, especially the white rat, only about 14 per cent of the latter dying as the result of inoculation. The mature dog is only slightly susceptible. Birds, especially pigeons, can be infected, but not easily. Frogs are completely resistant but toads are very susceptible.

The route by which the bacilli enter the body exerts an important influence in both experimental and natural infections. Subcutaneous inoculation is the method most commonly practiced in experimental work, and is almost

[10] Graham-Smith: Jour. Hyg., 1941, *41*:496.

[11] Minett and Dhandra: Indian Jour. Vet. Sci. Animal Husb., 1941, *11*:308.

[12] Preiss: Centralbl. f. Bakt., 1911, *58*:510; Nungester: Jour. Inf. Dis., 1929, *44*:73; *ibid.*, 1929, *45*:214; Bordet and Renaux: Ann. Inst. Pasteur, 1930, *45*:1.

uniformly fatal with the ordinary small laboratory animals. Feeding experiments show that administration of spore-free cultures even to highly susceptible animals is without result, owing to the destruction of the bacilli in the stomach. The feeding of spores, on the other hand, leads to infection of the more susceptible species, although not so certainly as subcutaneous inoculation; resistant species, such as swine, may be infected through the alimentary tract only with difficulty. Infection through the respiratory tract is possible in the experimental animal but is probably almost unknown in the lower animals under natural conditions.

In highly susceptible animals the disease is acute and runs a rapid course; the case fatality in cattle and sheep is about 80 per cent. It presents all the characteristics of a typical septicemia and local manifestations may be almost entirely absent. Enormous multiplication of the bacteria takes place in the blood and internal organs, and sections through the liver or spleen show the capillaries gorged with masses of bacteria. The spleen is of a deep-red color and greatly enlarged, hence the name splenic fever. The more resistant animal species do not develop this generalized infection, but the bacteria remain localized in an abscess or carbuncle and fail to spread through the body. This is the case with the dog and in some forms of infection in man. In this respect anthrax furnishes an illustration of the general rule that when a bacterial invasion meets slight resistance from the animal tissues an abundant multiplication of the bacteria occurs throughout the body, while the possession of high powers of resistance is accompanied by a pronounced local reaction. Man stands perhaps midway in susceptibility between the dog and the sheep.

Under natural conditions cattle and sheep are infected through the alimentary tract by swallowing spores while grazing in infected pastures. As has been pointed out, spores are able to retain their vitality in soil for a long period, and pastures once infected may infect cattle after a lapse of as many as thirty years. Hides imported from China and other countries where the disease prevails are not uncommonly contaminated with anthrax spores; in the United States several outbreaks of anthrax among cattle with some consequent cases of human infection have been traced to the overflowing of pasture land by streams receiving the drainage of tanneries.

Cattle may also occasionally be infected by direct contact through wounds, abrasions and other injuries to the skin; but alimentary infection is by far the most common. Anthrax has been experimentally transmitted to susceptible animals by biting flies of various species that had previously fed on animals dying from anthrax.[13] The bacilli persist in the insects for only a short time.

Pathogenicity for Man.—Three routes of infection of human beings are known: (*a*) through the skin, (*b*) through the respiratory tract and (*c*) through the alimentary tract. The bacillus is almost always transmitted to man through the agency of the lower animals rather than through other human beings. The persons most commonly affected are those having to do with cattle and their products, such as butchers, shepherds and

[13] Mitzmain: Hyg. Lab. Bull. No. 94, 1914. See also Kraneveld and Djaenoedin: Nederland. Indische Blad. Diergeneesk., 1940, 52:339.

herdsmen, handlers of hides, hair and fleeces. During the first World War less efficient preliminary disinfection of hides and bristles permitted the introduction of anthrax-contaminated articles from parts of Asia and South America, and a striking increase in anthrax occurred from the use of shaving brushes—the bacilli were isolated from brushes purchased in the open market in some instances. The bacilli are destroyed on such brushes by soaking in 10 per cent formalin at 110° F. for four hours. Laboratory infections, sometimes fatal, have been known to occur with pure cultures of the anthrax bacillus. The case fatality of anthrax in man is probably about 20 per cent.

Malignant Pustule.—The most common form of anthrax in the human subject is due to skin infection, and usually takes the form of a localized boil or abscess, which often heals spontaneously but may progress into a septicemic condition unless checked by incision or other surgical procedure. Owing to the relatively high resistance of man septicemia does not often occur, especially if the carbuncle be incised and thoroughly drained. Lesions of all sizes may be produced, from a minute pustule to a large abscess.[14]

Pulmonary Anthrax.—The pulmonary form of anthrax due to inhalation of the microorganisms is the most dangerous, although not the most common, variety of the disease in man. It is an occupational disease among those who handle and sort wools and fleeces and contract the infection by inhalation of spores set floating in the air from the infected material; pulmonary anthrax is known in England as "woolsorters' disease." It is characterized by many of the symptoms of pneumonia and often passes into a fatal septicemia.

Intestinal Anthrax.—The alimentary tract, although the usual path of infection in cattle, is very rarely so in man. A few instances are on record of intestinal anthrax contracted through the medium of spore-infected food. Such cases occur among workers with animal products, and have probably been due to lack of caution in handling food with uncleansed hands. Insufficiently cooked meat from anthrax-infected animals may also be a source of intestinal anthrax.

The "Toxicity" of the Anthrax Bacillus.—In typical anthrax septicemia bacilli are found in immense numbers clogging the capillaries, and it was early supposed that death resulted from an "internal suffocation" brought about by mechanical interference with the circulation. Death may occur, however, in the absence of great numbers of bacteria, and it might be supposed that the anthrax bacillus forms a toxin. All attempts to demonstrate the existence of either an extracellular or an intracellular anthrax toxin have, however, been unsuccessful, and, although all the probabilities are in favor of the existence of some such substance, the exact manner in which the anthrax bacillus damages the animal organism is as yet unknown. As indicated above, the virulence of *B. anthracis* is associated with the presence of a capsule and in this connection it may be noted that the d(-)glutamic acid polypeptide that is the capsular substance

[14] For a discussion of cutaneous anthrax see Hodgson: Lancet, 1941, *ii*:811; Gold: Arch. Int. Med., 1942, *70*:785.

is not hydrolyzed by the proteolytic enzymes of the body and is, in fact, excreted quantitatively by the rabbit.[15]

Immunity.—The cause of the high natural immunity to anthrax possessed by the dog, the fowl and certain other animals has been the object of much experimentation, but no clear-cut explanation has as yet been found. The body fluids of some species manifest bactericidal powers toward the anthrax bacillus, but there is no concurrence between the degree of immunity and the anthracidal power of the blood serum. The blood serum of the highly susceptible rabbit is strongly bactericidal outside the body, but anthrax bacilli injected into the circulation seem to multiply freely in the blood stream. Blood taken from the very resistant dog and fowl is practically devoid of bactericidal properties. It is likely that phagocytosis plays an important part in some cases; Hektoen[16] has shown that it is highly probable that the natural immunity of the dog is due to phagocytosis.

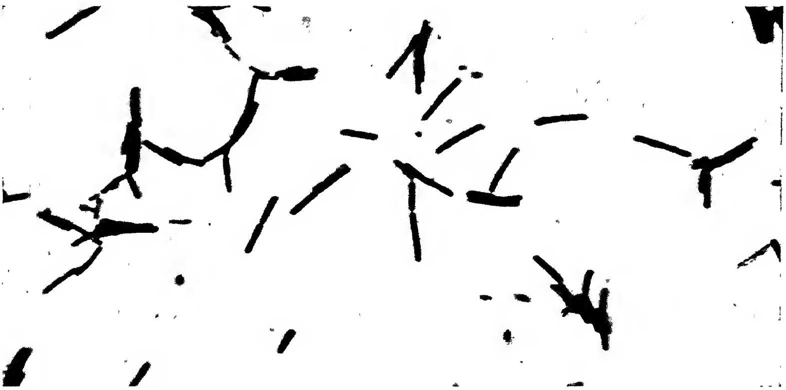


Fig. 116.—*Bacillus subtilis*, twenty-four-hour culture on nutrient agar. Crystal violet stain. No spores have formed as yet. Note the typical arrangement of the bacilli. × 1200.

Acquired immunity to anthrax is a consequence of the presence of living, virulent bacilli in the body; suspensions of killed cells or completely avirulent bacilli do not produce an immunity. Pasteur devised a method for vaccinating cattle and sheep against anthrax which is dependent on the subcutaneous inoculation of attenuated cultures. Two vaccines were used. The "first vaccine" consisted of bacilli grown at 42.5° C. for fifteen to twenty days and would not always kill guinea pigs though still fatal for white mice. After twelve days a second inoculation was made with the "second vaccine" of bacilli cultivated at the higher temperature ten to twelve days, which would kill guinea pigs but not rabbits. Following inoculation with these two vaccines, a fully virulent culture could be injected with impunity. In spite of some accidents due to the use of imperfectly

[15] Ivanovics: Ztschr. f. Immunitätsf., 1939, *96*:408.
[16] Hektoen: Jour. Amer. Med. Assn., 1906, *46*:1407.

standardized vaccines, this method of protective inoculation has proved, on the whole, of great practical value. In France 30,000 to 50,000 cattle and horses and 250,000 to 350,000 sheep are vaccinated annually. Active immunization of rabbits and guinea pigs can also be effected by the injection of attentuated cultures, but with much greater difficulty.

The simultaneous inoculation of anti-anthrax serum and a spore vaccine (Sobernheim's method) has been quite extensively practiced in the United States in districts where anthrax is prevalent, and here, too, occasional infections with the vaccine occur.

The serum of actively immunized animals contains specific protective substances, and inoculation with such antisera confers some degree of passive immunity. In the hands of Sobernheim,[17] Sclavo[18] and others such antisera have shown therapeutic value. Both Sclavo's and Eichorn's[19] sera have been used in human cases. More recently it has been found[20] that the

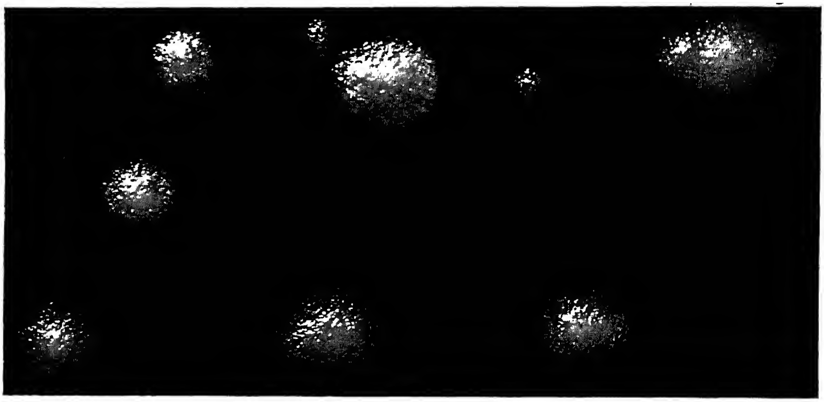


Fig. 117.—Colonies of *Bacillus subtilis* on nutrient agar. Twenty-four-hour culture. Note the resemblance to colonies of the anthrax bacillus. × 3.

antibody to the capsular substance is of particular importance but is not present in many of the commercial antisera. At the present time it is customary to treat anthrax cases with a combination of antiserum and arsenicals.[21]

The mechanism by which such antisera provide protection against infection is not certainly known. It may, perhaps, be reasonably assumed that anthrax immunity is a phagocytic immunity and that antisera act as sensitizing agents.

Bacteriological Diagnosis of Anthrax.—If the specimen is fresh and not grossly contaminated the anthrax bacillus may be found in gram-

[17] Sobernheim: Ztschr. f. Hyg., 1897, *25*:301; Centralbl. f. Bakt., I, 1899, *25*:840.
[18] Sclavo: Centralbl. f. Bakt., I, 1899, *26*:425.
[19] Eichorn: Jour. Agr. Res., 1917, *8*:37.
[20] Tomcsik and Ivanovics: Ztschr. f. Immunitätsf. u. Exp. Therap., 1938, *94*:28.
[21] *Cf.* Amer. Pub. Health Assn. Yearbook, Suppl. Amer. Jour. Pub. Health, 1935, *25*:No. 2; see also Cruickshank: Lancet, 1939, *ii*:681.

stained smears as a rule and is readily cultured on the usual nutrient media. Such a specimen may also be used for guinea pig inoculation, but it must be borne in mind that the sporulating obligate anaerobes will kill as rapidly as the anthrax bacillus. In any case the isolated culture must be tested for pathogenicity by animal inoculation. The animal will die in thirty-six to forty-eight hours after subcutaneous inoculation of a very small amount of culture. A gelatinous infiltration will be found at the site of inoculation and the tissues of the animal contain enormous numbers of the bacilli; smear of cut spleen, for example, will show many of the large gram-positive bacilli. This demonstration of pathogenicity is sufficient for identification since none of the aerobic sporulating rods that resemble the anthrax bacillus is pathogenic for the guinea pig and similar experimental animals.

A precipitation test, the Ascoli test or thermo-precipitation test, is sometimes used to detect anthrax contamination of hides or other tissues.

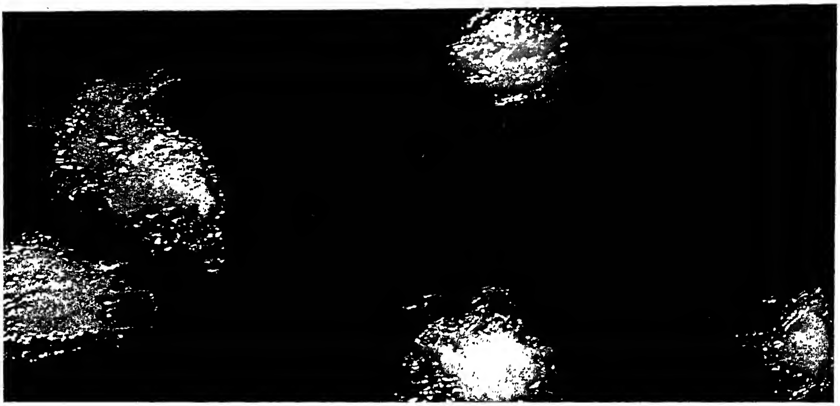


Fig. 118.—Colonies of *Bacillus mycoides* on nutrient agar. Twenty-four-hour culture. × 3.

The specimen is extracted with boiling water and the extract used as an antigen in a precipitin ring test with very high titer anthrax antiserum.

RELATED BACILLI

As indicated earlier, there are many species of aerobic sporulating bacilli closely related to and indistinguishable from the anthrax bacillus on any basis other than pathogenicity. The close morphological similarity led many of the earlier workers to describe "avirulent anthrax bacilli," "pseudo-anthrax bacilli," and "Bacillus anthracoides." There is, however, little basis for such differentiation. On the contrary, the vast majority of these bacilli are saprophytic soil forms and are commonly found as contaminants on plates because of the wide distribution of their spores in dust. Of these bacilli there are a number of well-defined types or species, the more common of which are:

Bacillus subtilis is the so-called "hay bacillus" which is very common and widely distributed; it is the type species of the genus *Bacillus*.

Bacillus vulgatus is sometimes called the "potato bacillus" and is likewise widely distributed in nature.

Bacillus mesentericus is also a common soil form and very similar to the other members of the group.

Bacillus megatherium is another of these forms; according to Ripple[22] this organism was originally named *Bacillus megaterium* (big rod) by DeBary and the name *Bacillus megatherium* (big animal) is a corruption which arose as a pun in some laboratories. It is larger than the above species.

Bacillus cereus is also larger and it, *B. megatherium*, and some other species are sometimes referred to as the "large-cell species" in distinction to the "small-cell species." *Bacillus mycoides*, sometimes called *Bacillus ramosus*, is very closely related to *B. cereus* and is thought by some to be the same species.

These forms can be distinguished from one another on the basis of details of spore formation, differential fermentations and the like, and constitute stable types. Immunological investigation has confirmed the homogeneity of these types also. The immunological specificity of the spores appears to be different from that of the cell substance, and Lamanna[23] has found that four main types of the small cell group can be distinguished though differentiation of the large cell group by this means is not so satisfactory. Sievers[24] has reported similar results in studies of the specificity of the cell substance.

The pathogenicity of these forms is very slight at best but *B. subtilis* is occasionally responsible for infection, particularly of the eye, and rarely may produce a septicemia in the immature animal. Other bacteria of this group are occasionally found to have feeble pathogenic powers. Heaslip[25] isolated an aerobic sporulating bacillus which he called *Bacillus tropicus* by the inoculation of mice with blood from persons suffering from a mild infection in Australia called "coastal fever." This bacillus has also been found there as a natural parasite of the rat and the bandicoot. It appears to be very similar to a bacillus described by Scott[26] many years before as *Bacillus seroficus*. *Bacillus alvei*, the cause of foulbrood of bees, is not pathogenic for man.

[22] Ripple: Arch. Mikrobiol., 1940, *11*:470.
[23] Lamanna: Jour. Inf. Dis., 1940, *67*:193, 203.
[24] Sievers: Jour. Bact., 1942, *43*:305.
[25] Heaslip: Med. Jour. Australia, 1941, *2*:536.
[26] Scott: Jour. Trop. Med. Hyg., 1912, p. 97.

CHAPTER 28

CLOSTRIDIUM—THE SPORE-FORMING ANAEROBES[1]

Family. *Bacillaceae*. Genus: *Clostridium*. Anaerobes or microaerophiles. Often parasitic. Rods frequently enlarged at sporulation, producing clostridium or plectridium forms. Type species: *Clostridium butyricum*.

The group of anaerobic sporulating bacilli includes a variety of forms. Some of these, the anaerobic nitrogen-fixing bacteria, the butyl alcohol and acetone-producing forms and others, have been discussed earlier (Chap. 5). Still others, however, are pathogenic for man and lower animals, and the more important of these forms—*Clostridium tetani*, *Clostridium septicum*, *Clostridium welchii*, *Clostridium novyi*, *Clostridium histolyticum*, *Clostridium chauvei*, *Clostridium botulinum* and the non-pathogenic but common species *Clostridium sporogenes*—will be considered here.

The status of these bacteria as parasites is open to some question. They occur in the soil, in particular abundance in manured soils, and are found in the intestinal tract of man and animals. *Cl. welchii*, for example, is uniformly present in human feces, and the tetanus bacillus is often found (in up to 40 per cent of specimens examined) in the feces of domestic animals. It has been assumed by some that these bacilli are parasitic and their presence in the soil is a consequence of contamination. Although their numbers are unquestionably greatly increased by manuring and other forms of contamination, some have been found in virgin soils. It is perhaps best to regard them as essentially saprophytic soil forms that are capable of maintaining themselves in the large intestine.

None of these bacteria possesses any marked ability to invade the body tissues by itself. *Cl. botulinum* is apparently incapable of setting up an infection, while others, such as the tetanus bacillus, produce local infections when aided by traumatic injury to the tissues and frequently by the presence of other bacteria. Still others, such as the bacilli associated with gas gangrene, show pronounced invasive properties when once established, but the initial invasion is made possible by other factors, usually trauma and the presence of other bacteria.

The pathogenicity of the anaerobic bacilli is, rather, attributable to their ability to form powerful exotoxins, a property which is curiously confined to these bacteria, the diphtheria bacillus and possibly the Shiga dysentery bacillus. In the case of botulism the toxin is preformed outside the animal body and, since it is unique in that it is resistant to the digestive enzymes, enters the body by way of the alimentary tract and absorption

[1] These and other anaerobic bacteria are considered at length by Weinberg, Nativelle and Prevot: *Les Microbes Anaerobies*. Masson et Cie. Paris. 1937. The serological relationships of these bacteria are reviewed by McCoy and McClung: Bact. Rev., 1938, 2:47.

into the tissues. In the other cases a focus of infection is established and the toxin formed at that point is disseminated through the body. In some instances, such as gangrene, an extensive local destruction of tissue occurs, but in general the diseases caused by these bacilli are essentially toxemias.

It will be clear from the foregoing considerations that infections with the sporulating anaerobes are not common under ordinary circumstances, for in most instances traumatic injury is a preliminary to infection. On the battlefield, however, such injuries are common, and tetanus and gangrene are not infrequent complications of war wounds. Such anaerobic wound infections were prominent in the first World War, perhaps as a consequence of battles fought over the heavily manured fields of France. In any case, the interest aroused resulted in considerable additions to knowledge of these infections, gangrene in particular.

Cl. histolyticum is microaerophilic, *i.e.*, it will grow in the presence of small amounts of oxygen, but the remainder of the forms considered here

MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF THE MORE IMPORTANT PATHOGENIC ANAEROBES

	Spores	Capsule	Motility	Proteolysis	Fermentations			Exotoxin
					Dextrose	Lactose	Sucrose	
Cl. tetani	spherical, terminal	−	+	−	−	−	−	+++‡
Cl. septicum	oval, subterminal	−	+	+sl*	+	+	−	++
Cl. welchii		+	−	+sl	+	+	+	++
Cl. novyi		−	+	+sl	+	−	−	+++
Cl. histolyticum		−	+	+	a†	−	−	+
Cl. sporogenes		−	+	+	+	−	−	−
Cl. chauvei		−	+	+sl	+	+	+	++
Cl. botulinum		−	+	±	+	±	−	+++

* sl: relatively slight; † a: acid; ‡ strong, moderate and weak.

are obligate anaerobes. They may be isolated in pure culture by picking colonies from a shake culture or from plates incubated in an anaerobic jar. All are large gram-positive rods, non-encapsulated with the exception of *Cl. welchii*, and motile by means of peritrichous flagella with the exception of *Cl. welchii*. Spores are usually of a greater diameter than the vegetative cells, and the spore-containing cells are spindle- or club-shaped. The spores of the tetanus bacillus are round and terminal, and those of the other bacilli oval and subterminal. Two general physiological types of anaerobic bacilli may be distinguished, the one predominantly fermentative or saccharolytic and the other predominantly proteolytic. These and other characteristics are summarized in the accompanying table.

Large amounts of volatile organic acids are produced in the fermentation of carbohydrates by these bacilli; they differ in this respect from the other pathogenic bacteria which produce non-volatile acid, *i.e.*, lactic acid, for

the most part. Amino acids are vigorously attacked by the obligate anaerobes; some are "fermented" to organic acids,[2] while others are mutually oxidized and reduced, a process that has been discussed elsewhere (p. 111).

TETANUS (CLOSTRIDIUM TETANI)

Tetanus is a disease of man and animals characterized by spasms of the voluntary muscles. The spasms are often most marked in the muscles of the jaw and neck, hence the name "lockjaw." The tetanus bacillus was first described in 1884 by Nicolaier,[3] who observed it in the pus taken from mice and other animals that had died after subcutaneous inoculation with small quantities of soil. Kitasato[4] isolated the microorganism in pure culture and demonstrated its causal significance. He also proved the inability of the tetanus bacillus to invade the blood stream and showed the disease to be

Fig. 119.—*Clostridium tetani* in pure culture. Young, actively growing culture showing beginning spore formation. Note the refractile, unstained spores, the drumstick appearance when these are attached to the cells, and the tendency of the vegetative cells to remain attached end to end. Fuchsin; × 1150.

an intoxication.[5] In 1890 von Behring and Kitasato[6] laid the basis for antitoxic therapy in their discovery of diphtheria and tetanus antitoxin.

Morphology.—Individual tetanus bacilli are slender, motile (20 to 30 peritrichous flagella), gram-positive, sporulating rods with rounded ends. Their common dimensions are 0.3 to 0.5 μ in width and 2 to 5 μ in length, but vegetative filaments of much greater length occur. The shorter forms are usually straight; filaments tend to curve in an undulating manner. Short chains of rods may occur. The spore is spherical and terminal and larger in diameter than the vegetative cell; spore-containing cells have a

[2] Such as the conversion of glutamic acid to butyric and acetic acids, carbon dioxide, ammonia and hydrogen. *Cf.* Barker: Jour. Bact., 1938, *36*:322.

[3] Nicolaier: Deut. med. Wchnschr., 1884, *10*:842.

[4] Kitasato: Ztschr. f. Hyg., 1889, *7*:225.

[5] *Ibid.*, 1891, *10*:267.

[6] von Behring and Kitasato: Deut. med. Wchnschr., 1890, *16*:1113.

characteristic drumstick appearance (Fig. 119). Isolated colonies in deep dextrose agar have a woolly appearance and may either be flocculent or have an opaque center. Surface colonies are flat, rhizoid, or even feathery, and frequently exceed 1 mm. in diameter. Later the centers may become slightly raised. Colonies on blood agar show hemolysis.

Physiology.—The tetanus bacillus develops in plain or dextrose broth and in brain, meat, agar and gelatin media from which the air has been expelled by heating and excluded by some form of seal. If the depth of media is adequate, say 10 to 12 cm., no special seal is required, especially for the more viscous media. Growth occurs between 14° and 43° C.; the optimum temperature is 37° C.

The growth of *Cl. tetani* is influenced greatly by the presence of associated microorganisms. In sugar-free media it may be grown in mixed cultures upon the surface of culture media in contact with air through the absorption of oxygen by the associated aerobes. But in dextrose broth the growth of the tetanus bacillus in mixed culture is likely to be inhibited by acid formation due to the associated bacteria. Sugar-free media, such as meat infusion peptone broth or deep meat or brain media, are therefore preferable for initial culture from contaminated material. Pure cultures may be isolated from these initial cultures by deep agar or surface culture methods.[7] It has been supposed that an initial heating of the contaminated material simplifies isolation of the tetanus bacillus by destroying the vegetative cells of other microorganisms that may be present; this method may, however, leave the spores of the tetanus bacillus still badly mixed with those of other aerobic and anaerobic bacteria, some of which may be more resistant than the tetanus spores.

In pure culture, dextrose stimulates growth as in the case of the other anaerobes, although dextrose and other carbohydrates are not fermented by *Cl. tetani*. Sporulation is not inhibited by carbohydrates, however, as with the fermentative anaerobes; on the contrary, sporulation of the tetanus bacillus is accelerated in dextrose broth.

Growth in gelatin stab is slow at 22° C., but in ten days or so the characteristic inverted fir-tree type of growth is apparent. Incubated at 37° C. for two to three days, gelatin cultures usually fail to stiffen in the refrigerator. Coagulated proteins, such as blood serum and egg white, are very slowly liquefied. Deep brain and meat media may be slightly softened, but never digested fully; after several weeks a slight darkening occurs near the surface exposed to air. The tetanus bacillus is, therefore, only weakly proteolytic. Amino acids are, however, utilized readily but not by paired oxidation-reduction; glutamic acid, aspartic acid and serine, for example, are attacked directly with the formation of CO_2, NH_3 and acetic and butyric acids.[8] Both amino acids and carbon compounds are readily dehydrogenated.[9] In litmus milk there is reduction of the indicator and sometimes a slight precipitation of the casein. Nitrates are not reduced to nitrites, but hydrogen sulfide and indol are produced.

[7] *Cf.* Reed and Orr: Proc. Soc. Exp. Biol. Med., 1941, *48*:535; War Med., 1941, *1*:493.
[8] Clifton: Jour. Bact., 1942. *44*:179.
[9] Pickett: Jour. Biol. Chem., 1943, *151*:203.

The tetanus bacillus has been grown in synthetic media and its growth requirements are relatively complex, including the amino acids arginine, histidine, tyrosine, valine, leucine, isoleucine and tryptophane; the bacterial vitamins riboflavin, pantothenic acid, thiamin, "folic acid," biotin, pyridoxine, and nicotinic acid; the purines adenine and uracil; and oleic acid.[10] The formation of toxin is inhibited by iron and best yields are secured when as much iron as possible is taken out of the medium,[11] though undoubtedly very small amounts are required. The production of toxin for the preparation of toxoid is now being carried out in the peptone-free casein hydrolysate medium of Mueller and Miller.[12]

Spore formation begins in about two days at 37° C. and in eight or ten days at room temperature. The spores are highly resistant and when protected from light and heat remain viable for years. Theobald Smith found a number of strains that resisted steaming at 100° C. for forty to sixty minutes. Five per cent phenol is said to destroy tetanus spores in ten to twelve hours; the addition of 0.5 per cent hydrochloric acid may reduce the time to two hours. Mercuric chloride, 1:1000, kills in two to three hours, with 0.5 per cent hydrochloric acid in thirty minutes. One per cent silver nitrate kills in one minute.

Antigenic Structure.—The tetanus bacillus is antigenically heterogeneous, and a number of types, designated by Roman numerals, have been described.[13] At present ten in all are known.[14] Both somatic and flagellar antigens appear to be involved, the former of a group and the latter of a specific nature. Types I and III have been found most commonly in the United States, England and France, Type V in China. The toxin formed by these types is immunologically identical and the subdivision into agglutination types does not appear to have any practical significance.

Tetanus Toxin.—As indicated elsewhere, the potent soluble toxin produced by the tetanus bacillus is of dual nature; *tetanospasmin* is the portion that affects the nervous tissue and *tetanolysin* is a hemolysin. The former is by far the more important; there is no indication that tetanolysin has any significance in connection with the ordinary symptom-complex. Liquid cultures are usually highly toxic; 5×10^{-6} cc. may be fatal to a mouse weighing 10 gm. Hall[15] repeatedly produced toxin of which the guinea pig MLD was rarely more than 0.0001 cc. and usually less. Ruediger[16] was equally successful, and in one instance produced tetanus toxin of such strength that 1 cc. would have sufficed to kill 50,000 guinea pigs.

The toxin is filterable and may be freed from bacteria by filtrations through Berkefeld, Chamberland and similar filters. In aqueous solution the toxin is highly unstable to heat and light and must be stored in a cold dark place. It is apparently protein in nature, or at least has not as yet

[10] Mueller *et al.*: Jour. Bact., 1942, *43*:763; *ibid.*, 1943, *46*:559, 563.

[11] Mueller and Miller: Proc. Soc. Exp. Biol. Med.. 1940, *43*:389.

[12] Mueller and Miller: Jour. Immunol., 1943, *47*:15.

[13] *Cf.* Gunnison: Jour. Immunol., 1937, *32*:63.

[14] The tenth type has been described by Maclennan: Brit. Jour. Exp. Path., 1939, *20*:371.

[15] Hall: Univ. Calif. Pub. in Path., 1913, *2*:97.

[16] Ruediger: Philippine Jour. Sci., 1915, *10*:31.

been separated from protein, and is destroyed by proteolytic enzymes and hence is ineffective when given by mouth. It may be precipitated with ammonium sulfate and in the dry state retains its potency for a long time. It is an excellent antigen and gives rise to high titer antitoxic sera.

As indicated above, tetanus toxin is one of the most potent poisons known, and its toxicity far exceeds that of alkaloids and other substances generally regarded as highly poisonous. Its potency is not known with any precision because, so far as is known, it has never been prepared in a state of purity. Ammonium sulfate precipitates were found by Brieger and Cohen[17] to kill white mice in doses of 5×10^{-8} gm., and the preparations of London and Aristovsky[18] killed mice in amounts of 2×10^{-8} gm. Eaton's[19] purified material was lethal for 500 gm. guinea pigs in amounts as small as 18×10^{-6} gm.

The amount of toxin required to kill different animals varies considerably, as the following comparative figures from Knorr[20] show:

	Destroyed by
1 gram of horse	x toxin
1 gram of goat	2x toxin
1 gram of mouse	13x toxin
1 gram of rabbit	2000x toxin
1 gram of hen	200,000x toxin

Cold-blooded animals are not susceptible to tetanus toxin. An incubation period is always observed, and this cannot be shortened beyond a certain minimal limit (in the mouse eight hours), even with large doses. Otherwise the incubation period and the time of death are inversely proportional to the amount of toxin injected.

The Dissemination of Tetanus Toxin in the Body.—There are two views concerning the pathway by which tetanus toxin reaches the central nervous system. According to the experimental evidence of Meyer and Ransom,[21] tetanus toxin is absorbed by the end-organs of the motor nerves and travels to the ganglion cells of the central nervous system, not by way of the blood or lymph channels, but along the axis-cylinders of the peripheral nerves. The time consumed in this passage represents the larger part of the incubation period. The toxin may circulate for a time in the blood, but the only path to the central nervous system lies along the axis-cylinders of the motor nerve tract. Intravenous injection of toxin into experimental animals causes only general tetanus, according to Ransom's later reiteration of this view.[22] A cut nerve takes up the toxin very slowly and a degenerate nerve not at all. Section of the spinal cord prevents the toxin from reaching the brain. Meyer and Ransom believed that the spinal ganglion of the sensory nerve presents a barrier to the advance of the toxin, and that for this reason sensory nerves are unable to conduct the toxin. The remarkable excitation

[17] Brieger and Cohen: Ztschr. f. Hyg., 1893, *15*:8.
[18] London and Aristovsky: Compt. Rend. Soc. Biol., 1917, *80*:756.
[19] *Cf.* Eaton: Bact. Rev., 1938, *2*:3.
[20] Knorr: Münch. med. Wchnschr., 1898, *45*:321.
[21] Meyer and Ransom: Arch. f. Exp. Path. u. Pharm., 1903, *49*:369.
[22] Ransom: Lancet, 1917, *ii*:928.

of the motor cells of the spinal cord that is observed in tetanus is not accompanied by characteristic lesions.

Although long generally accepted, this view has been challenged in recent years by Abel and his co-workers,[23] who propose the following theory as in accord with their experimental findings: The toxin exhibits both a central and a peripheral action, each of which may be demonstrated independently of the other. The central effect, which is characterized by reflex motor convulsions, is due to the poisoning of the motor nerve cells of the spinal cord, medulla and pons; the peripheral effect, recognized as the unremitting rigidity of voluntary muscles, results from the fixation of the toxin upon the motor end-organs. Following subcutaneous or intramuscular injection the toxin is absorbed by the lymphatics and distributed to the central nervous system by way of the arterial circulation. Normal tonicity of the motor end-organs seems to be essential to the development of tetanic rigidity; neurotomy results in an immediate, sharp depression of the tonicity of the myoneural junctions, hence they are not responsive to the influence of tetanus toxin.

Abel's views have not been generally accepted in spite of their support by convincing experimental evidence which cannot be reviewed here. Other experimental data have recently been presented in support of the theory of the axis-cylinder pathway,[24] and as yet no conclusion may be drawn.

Tetanus toxin possesses a strong affinity for the cells of the central nervous system, as evidenced by the now classic experiments of Wassermann and Takaki.[25] A mixture of toxin and brain substance can be injected into an animal without producing any toxic effect, the toxin apparently entering into a firm combination with some ingredient of the nerve substance. Not only the central nerve cells, but to some extent other tissue cells, are able to bind tetanus toxin. Subcutaneous inoculation is less likely to result fatally than direct inoculation into nerve tissues because some of the toxin is bound and prevented from reaching the highly sensitive nerve cells.

Pathogenicity.—Tetanus is essentially an intoxication. The bacilli set up a localized infection and the toxin formed there is disseminated through the body and gives rise to the symptom-complex characteristic of the disease. Bacillemia may occur very rarely, however, and has been produced experimentally.[26] The bacilli generally gain entrance to the tissues by means of a deep, dirty wound which may be relatively small, so small sometimes as to escape serious attention. The widespread occurrence of the tetanus bacillus would seem out of harmony with the relative infrequency of tetanus infection but, it may be noted, mere introduction of the bacillus into the body is not sufficient to produce the disease; the microorganisms must find favorable conditions for proliferation at the site of penetration. Experimentally, pure cultures of vegetative cells or spores that have been freed

[23] In a series of publications beginning in 1934. Reference may be made to the ninth paper by Abel, Warfield and Chalian: Bull. Johns Hopkins Hosp., 1938, *63*:373.

[24] *Cf.* Friedemann, Hollander and Tarlov: Jour. Immunol., 1941, *40*:325.

[25] Wassermann and Takaki: Ber. klin. Wchnschr., 1898, *35*:5.

[26] Piringer: Centralbl. f. Bakt., I Abt., Orig., 1941, *147*:35.

from toxin cannot germinate in uninjured tissues, but simultaneous inoculation with common saprophytes, such as *Bacterium prodigiosum*, or with irritant chemicals, such as calcium salts or lactic acid, enables the bacilli to grow and form toxin. As indicated elsewhere (p. 90), a sufficiently low oxidation-reduction potential is necessary for the germination of tetanus spores, and it is not unlikely that the potential of normal tissues is too high to allow germination but is reduced by injury.

Tetanus of the newborn or *tetanus neonatorum* is a consequence of infection of the umbilicus through septic midwifery. It is especially common among the Negroes of the southern states and in other races living under unhygienic conditions.

The tonic spasms characteristic of tetanus usually begin at the site of infection, and the initial symptoms may include headache and stiffness of the neck. The spasms may remain localized in mild infections, but usually they are general and involve the whole somatic muscular system. Postmortem findings are insignificant; other than a moderate congestion, the organs show no pathological changes and the initial lesion may, of course, be inapparent or small.

The incubation period of tetanus is variable and may range from two to fifty days. The case fatality is inversely related to the incubation time; it may be as high as 70 to 80 per cent or as low as 15 to 20 per cent. Death, if it occurs, follows relatively soon after the appearance of symptoms; the dictum of Hippocrates, "such persons as are seized with tetanus die within four days, or if they pass these they recover," still stands. When the disease has a prolonged incubation period, a less sudden development of symptoms, and consequently more favorable prognosis, it is sometimes termed "chronic."

Lower Animals.—Tetanus is not a rare affection in the horse, the symptoms and course of the disease being similar to the disease in man. Cattle, sheep and hogs are less commonly affected. Experimentally, tetanus can be produced in mice and guinea pigs by the inoculation of spores introduced on splinters of wood, and also by injection of toxin. The feeding of animals with tetanus bacilli, spores or toxin is without effect. Tetanus differs from most other infectious diseases in that the diseased animal is not an appreciable factor in the spread of infection. A normal horse apparently may distribute tetanus spores quite as widely and freely as a horse sick with tetanus.

Immunity.—As indicated earlier, tetanus toxin is an excellent antigen and high titer antitoxic sera may be prepared. Horses are good producers of antitoxin and are immunized by toxin-antitoxin mixtures followed by toxin alone. In France toxoid is used for the first injections.

The Standardization of Antitoxin.—The antiserum is standardized according to the method of Rosenau and Anderson,[27] in which the American immunity unit is defined as "ten times the least quantity of antitetanic serum necessary to save the life of a 350 gm. guinea pig for ninety-six hours against the official test dose of a standard toxin furnished by the Hygienic Laboratory of the Public Health and Marine Hospital Service." The official

[27] Rosenau and Anderson: U. S. Pub. Health Serv. Bull. No. 43, 1908

test dose is about 100 guinea-pig MLD's of a precipitated standard toxin. It may be noted that the L_0 rather than the L_+ endpoint is determined. Thus the tetanus antitoxic unit has slightly more than 10 times the experimental protective power of the diphtheria antitoxic unit. An antitoxic unit strength of 900 per cc. may be attained exceptionally. Practice has been different in other countries, however, and the Permanent Committee on Standardization of the Health Organization of the League of Nations found that 1 German unit, 66 American units and 3750 French units were equivalent. It has been agreed that the international unit shall be one-half of the American unit.[28]

The appearance of multiple zones of precipitation has interfered with the application of the Ramon flocculation method to tetanus toxin, toxoid and antitoxin. Goldie, Parsons and Bowers[29] have found, however, that a specific flocculation zone occurs with refined enzyme-treated antitoxin.

The antitoxin deteriorates with time, of course, the most important factor being the temperature at which it is stored. Amies[30] has found that during storage for one year no potency is lost, at room temperature the loss is 7 to 9 per cent, and at 37° C. it is 44 to 47 per cent.

The Prophylactic Use of Antitoxin.—In veterinary practice, tetanus antitoxin has been used prophylactically with a high degree of success, as the references compiled by MacConkey[31] show. Vaillard collected the statistics from 1898 to 1906 of eight veterinary surgeons who inoculated 13,124 animals after operations or accidental wounds, without the occurrence of a single case of tetanus. During the same time two veterinary surgeons alone saw 139 cases of tetanus among animals which did not receive the treatment. The figures of Nocard and Labat added to Vaillard's data cover the cases of 16,917 animals recieving prophylactic injections; among them only one horse had tetanus. In this case the antitoxin was given five days after the wound and the attack was mild.

It is probable that in many cases tetanus is averted in man by the prophylactic use of antitoxin. Even though the disease is not prevented the incubation period is delayed and the disease may be very mild or remain localized. Precise statistical evidence involving comparable series of controls is not, of course, available, but the experience of the first World War was that with increased use of tetanus antitoxin there was not only a lower incidence of the disease but an increase in mild and chronic cases. Passive immunization provides antibody in the circulating blood which combines with toxin and renders it harmless. The avidity of the nervous tissue for tetanus toxin is very great, however, and symptoms may appear in spite of the presence of circulating antibody. Passive immunity is transient, of course, and repeated injections of antitoxin at six- to seven-day intervals must be made as long as danger of infection remains.

The Therapeutic Use of Antitoxin.—Tetanus antitoxin appears to have

[28] *Cf.* Prausnitz: *Memoranda on the International Standardization of Therapeutic Sera and Bacterial Products.* League of Nations Health Organization. 1929.

[29] Goldie, Parsons and Bowers: Jour. Inf. Dis., 1943, *71*:212.

[30] Amies: Brit. Med. Jour., 1941, p. 709.

[31] MacConkey: Brit. Med. Jour., 1914, *ii*:609.

but limited therapeutic value. It is obvious, of course, that symptoms result from damage to the nerve tissue and the administration of antitoxin will neither repair such damage nor displace the more avid nerve tissue already in combination with toxin. Reports on the therapeutic value of antitoxin are conflicting; some workers contend that intrathecal administration either alone or combined with intramuscular injection has a beneficial effect. The records of Cook County Hospital in Chicago, however, show that the therapeutic use of antitoxin has not reduced the mortality from tetanus there.[32]

Active Immunization.—In recent years some emphasis has been placed upon active immunization against tetanus, particularly by the French workers. In a summary of the results of twelve years' experience with active immunization of horses and men with formol toxoid, Ramon[33] states that in one cavalry unit in which tetanus was endemic more than 50,000 horses were immunized over a ten-year period and tetanus has practically disappeared, and in a million and a half human beings immunized with toxoid no case of tetanus has occurred.

With the beginning of the second World War, active immunization against tetanus was adopted by the armed services of France, Britain and the United States. Formol toxoid has been used for the most part as an immunizing preparation but alum precipitated toxoid is regarded by many as the preferred preparation[34] and is used by the United States Navy. No international standard exists for tetanus toxoid; the United States Army specified that the toxin contain at least 10,000 guinea pig MLD's per ml. and be detoxified with 0.4 per cent formalin; the final preparation must be atoxic for guinea pigs in 5 ml. amounts, and pigs receiving 1 ml. as an immunizing dose must be able to withstand 10 MLD of toxin at the end of six weeks.[35] More rapid methods of assay using mice have been suggested by Greenberg, Morrell and Gibbard[36] and by Koerber.[37] For immunization three doses of 1 ml. each are given at intervals of three weeks and a stimulating dose one year later. The value of more than two doses is illustrated by Evans[38] who found that the mean antitoxin titer after two inoculations was 0.35 IU (International Units) but with a third inoculation ten months later the mean titer rose to 10 IU and eighteen months later was still 0.37 IU.

In practice this immunization appears to be highly effective. From the initiation of immunization in the United States Army in 1941, there were only four cases of tetanus, and those in unimmunized persons, in the following two years. British experience in the Middle East war zone similarly indicated an effective immune response, though in most cases only two doses were given.[39] The success of active immunization, coupled with the low incidence of untoward reactions (reported to be 1 in 10,000 immunizations with improved toxoid), has suggested its more general application in

[32] Calvin: Jour. Amer. Med. Assn., 1930, *94*:1977.
[33] Ramon: Rev. Immunol., 1939, *5*:477.
[34] Jordan and Halperin: War Med., 1941, *1*:227.
[35] Long: Amer. Jour. Pub. Health., 1943, *33*:53.
[36] Greenberg, Morrell and Gibbard: Jour. Immunol., 1943, *46*:333.
[37] Koerber: Jour. Immunol., 1943, *46*:391, 411.
[38] Evans: Lancet, 1943, *ii*:316.
[39] Boyd and Maclennon: Lancet, 1942, *i*:745.

civil life. In France, where active immunization to tetanus has been of more and earlier interest than elsewhere, it has been made compulsory as with diphtheria immunization.[40] In this country considerable interest has attached to combined tetanus and diphtheria immunization of children[41] but this has not found general application as yet.

GASEOUS GANGRENE

Gaseous gangrene is a syndrome often following dirty, lacerated wounds, especially those involving fractures. It is a characteristic complication of war wounds, and present knowledge of this affection was largely developed during the first World War. But this disease is by no means so rare in civil life as was formerly thought. The increased number of injuries in automobile accidents is responsible for many cases of gangrene. Men injured about railroad tracks, either employees or vagrants, seem particularly prone to develop gaseous gangrene if not properly and promptly treated. Certain forms of peritonitis, appendicitis, intestinal obstruction, puerperal sepsis

OUTSTANDING PATHOLOGICAL CHANGES IN GUINEA PIGS INOCULATED WITH ANAEROBES OR THEIR TOXINS
(Hall)

	Edema	Emphysema	Congestion	Histolysis
Cl. tetani	−	−	+	−
Cl. septicum	+	++	+++	+
Cl. welchii	++	+++	+	+
Cl. novyi	+++	−	−	−
Cl. sordellii	+++	−	+	−
Cl. histolyticum	−	−	++	+++
Cl. chauvei	+	++	+++	+
Cl. botulinum	−	−	+	−

and postoperative infections (particularly laparotomy) are etiologically closely related to it.

In the fulminating form of gaseous gangrene the muscles become filled with gas and with a serosanguineous exudate depending for its character upon the associated microorganisms, for this disease is nearly always a mixed infection of aerobes and anaerobes of several species. The accompanying table from Hall[42] shows the chief pathological changes in guinea pigs inoculated with various anaerobes or their toxins.

Weinberg and Séguin[43] studied 126 cases of gangrenous wound infection other than tetanus. Of these, 35 were phlegmonous and 6 of them yielded only aerobic bacteria; the remainder contained anaerobic bacilli. Thus aerobes are apparently able to produce gaseous phlegmons, but the number of such cases is small; gaseous infections are predominantly anaerobic and

[40] Bull. Office Internat. d'Hyg. Publique, 1940, *32*:748.
[41] *Cf.* Bigler and Werner: Jour. Amer. Med. Assn., 1941, *116*:2355.
[42] Hall: Jour. Bact., 1928, *15*:17.
[43] Weinberg and Séguin: *La Gangrène Gazuse.* Masson et Cie. Paris. 1918.

the rôle of aerobes is, as a rule, only accessory. Of the 120 cases of anaerobic infection, 90 contained both aerobes and anaerobes; 70 contained more than 1 anaerobe and 50 but a single anaerobic species. The frequency with which the various anaerobic species were found is summarized in the accompanying table.

Clearly, then, the bacteriology of gaseous gangrene is complicated and while, as stated by Weinberg and Séguin, there is in effect a typical form of gaseous gangrene, (*a*) it is not always produced by the same microorganism; (*b*) it is frequently caused by several associated agents; (*c*) it is often the complex result of the combined action of these principal anaerobic bacilli with various other bacteria which play an indeterminate accessory rôle.

The more important of the anaerobic bacilli associated with gaseous gangrene are discussed in the following sections.

THE FREQUENCY OF OCCURRENCE OF ANAEROBIC BACILLUS SPECIES IN GASEOUS GANGRENE

Species	Mixed Anaerobic Flora		Pure Anaerobic Flora	
	Cases	Per Cent	Cases	Per Cent
Cl. perfringens (*welchii*)	91	72	37	74
Cl. sporogenes	34	27	2	4
Cl. edematiens (*novyi*)	33	26	6	12
Cl. fallax	26	21	3	6
Cl. septicum	12	9.5	1	2
Cl. tetani	11	8.7	—	—
Cl. histolyticum	8	6.3	—	—
Cl. aerofoetidum	5	3.9	1	2
Cl. putrificum	2	1.6	—	—
Cl. bifermentens (*sordellii*)	2	1.6	—	—
Cl. tertium	1	0.8	—	—
Bacillus II of Ghon and Sachs	1	0.8	—	—

1. The Vibrion Septique, Clostridium Septicum

In 1877 and 1881 Pasteur,[44] while studying anthrax, produced asepticemia in rabbits and guinea pigs by the inoculation of putrid blood from a cow. The affection could be communicated from individual to individual, and a sporulating, motile, rod-shaped anaerobe, considered by him "one of the vibrions of putrefaction" (the actively motile bacilli sometimes appear to be curved), was regarded as the cause of the septicemia and named "vibrion septique."

In 1881 Koch[45] described the pathological effects of a microorganism which he declared identical with the vibrion septique of Pasteur. But this bacterium failed to produce a septicemia in guinea pigs, and since its pathogenic effects were limited largely to the site of inoculation, Koch designated it "the bacillus of malignant edema."

[44] Pasteur: Bull. Acad. Med., 1877, p. 781; 1881, 2s., *10*:176.

[45] Koch: Mitteil. aus dem kais. Gesundheitsamte, 1881, *1*:1.

Neither Pasteur's nor Koch's description would suffice now to identify with certainty the microorganisms in question. Fortunately, the original strain of Pasteur's "vibrion septique" has been maintained in France, so that its outstanding characteristics are well known. The lack of any such legacy from Koch in Germany, due to his failure to recover cultures, has led to an all but interminable discussion as to the properties of the "true bacillus of malignant edema." The vibrion septique is now generally known as *Cl. septicum.*

Morphology.—The vibrion septique is a gram-positive, sporulating, spindle-shaped rod, or filament, and in young cultures motile, with many peritrichous flagella. The ends are slightly rounded and the spores, which are oval, are usually median and swell the vegetative cell into a clostridium previous to their release. Spores are formed only in media not containing fermentable carbohydrate in excess. The long chains and filaments of these microorganisms which occur on the visceral surfaces of infected guinea pigs are of high differential value. Capsules have never been observed. Deep colonies in 1 per cent agar are transparent or semitransparent. Hemolysis occurs on blood agar.

Fig. 120.—*Clostridium septicum* from pure culture. The tendency to form elongated vegetative cells is apparent. Fuchsin; × 1050.

Physiology.—*Cl. septicum* is a strict anaerobe and develops readily in deep brain or tissue media, producing gas rather abundantly. These media are not discolored even in the presence of metallic iron. Gelatin is liquefied, but coagulated serum and other proteins are not digested or blackened. Hydrogen sulfide is produced but indol is not. Dextrose, levulose, galactose, maltose, lactose and salicin are fermented; media not containing one of these sugars support only slight growth. Sucrose, inulin, mannitol and dulcitol are not fermented. The fermentation of salicin and non-fermentation of sucrose allow the biochemical differentiation of *Cl. septicum* and *Cl. chauvei,* for the latter does not ferment salicin but does ferment sucrose.

Antigenic Structure and Toxin.—Strains of *Cl. septicum* are immunologically related but distinct. Four groups have been distinguished on the basis of the somatic antigen, and subdivisions may be made by the flagellar antigen. These bacilli are immunologically related to *Cl. chauvei*. The toxin formed, however, is specific, but a relatively weak lethal agent. The MLD for mice is about 0.005 cc. When injected into animals it produces a gelatinous edema and some local necrosis of the tissues. According to Kellaway, Reid and Trethewie[47] the toxin has a specific cardiac action in the cat and rabbit, producing a fall in systemic and a rise in venous blood pressure, in the cat a specific constriction in the pulmonary and coronary circulations, with edema of the lungs and loss of fluid from the circulation.

Pathogenicity.—*Cl. septicum* does not occur in gaseous gangrene of man as frequently as some of the other anaerobic bacilli but has been found in such affections both alone and in mixed cultures. It has been recovered from gaseous infections in cattle and may be one of several microorganisms responsible for blackleg, usually considered a specific disease due to *Cl. chauvei*. It has also been found in gaseous infections of hogs and other domestic animals. Experimentally the vibrion septique is strikingly pathogenic for chickens, pigeons, rabbits, guinea pigs, rats and mice. In such animals the bacteria develop rapidly, producing gas and a reddish, serous edema. They invade the adjacent tissues and the circulation, producing a septicemia which is usually fatal within twenty-four to forty-eight hours; sublethal doses do not produce any reaction. Impression smears from the tissues, and especially from the liver, usually show elongated filaments or chains as contrasted with the single bacilli found in animals killed with *Cl. chauvei*.

Antitoxic sera which are prophylactic and, to some degree, curative may be prepared by injection of *Cl. septicum* toxin into horses. The antisera do not have the high antitoxin content that is found in antitetanic sera. Polyvalent commercial sera for prophylactic and therapeutic use in wound infections often contain antibodies to *Cl. septicum*.

2. Clostridium Welchii (Clostridium Perfringens)

Clostridium welchii was first cultivated by Achalme[48] and supposed by him to be the cause of articular rheumatism. In 1892 Welch and Nuttall[49] isolated this bacillus from the foamy organs of a cadaver and called it *Bacillus aerogenes capsulatus*. Found by Fränkel[50] the following year, it was designated *Bacillus phlegmonis emphysematosae*, and in 1897 Veillon and Zuber called it *Bacillus perfringens*. Sometimes called Fränkel's bacillus in Germany, *Cl. perfringens* in France and *Cl. welchii* in English-speaking countries, it is designated *Cl. perfringens* by Bergey.

Morphology.—*Cl. welchii* is a plump, non-motile, gram-positive rod of variable length, occurring in chains and singly. Capsules are usually present in preparations made from the organs or body fluids. Spores are

[47] Kellaway, Reid and Trethewie: Australian Jour. Exp. Biol. Med. Sci., 1941, *19*:297.
[48] Achalme: Compt. Rend. Soc. Biol., 1891, *13*:651.
[49] Welch and Nuttall: Bull. Johns Hopkins Hosp., 1892, *3*:81.
[50] Fränkel: Centralbl. f. Bakt., 1893, *13*:13.

formed sparingly and only in the absence of fermentable carbohydrates; they are centrally located, rarely subterminal, and do not swell the vegetative cell in which they are formed. Isolated colonies in deep agar are compact, opaque, white or grayish white biconvex disks. On blood agar the round, smooth, opaque, entire-edged colonies are relatively large, 2 to 5 mm. in diameter, and surrounded by a zone of hemolysis.

Welch's bacillus is a strict anaerobe and grows readily in deep brain, meat infusion broth, agar and gelatin media. Its growth in sugar-free media is greatly restricted. Optimum conditions for growth are provided by media containing fermentable carbohydrates, but such cultures are often short-lived because of the lack of spore formation and the destructive action of the formed acids on the vegetative cells. Brain and meat media are not blackened normally, but the presence of metallic iron produces a distinct discoloration. Gelatin is liquefied but coagulated serum or egg is not

Fig. 121.—*Clostridium welchii* from pure culture. Note the relatively smaller size of these bacteria and the central spores. Fuchsin; × 1050.

digested. Hydrogen sulfide is produced, indol is usually said to be negative but its formation is uncertain.

Acid and gas are produced in glucose, maltose, lactose and sucrose; neither mannitol nor salicin is fermented; some strains ferment inulin and some glycerol. Broth cultures containing fermentable sugars become markedly turbid with abundant gas formation, and many cultures, possibly all at certain stages, become stringy and viscid. Milk is fermented with a characteristic "stormy" evolution of gas, followed by coagulation of the casein due to acid formation, and the curd is shortly torn to shreds by the continued evolution of gas within. The curd is not digested. This "typical" reaction is considerably modified by incomplete anaerobiosis; gas production may be slow and the solid curd is torn only slightly if at all. Under optimum conditions 3.8 times the volume of the milk may be evolved as gas; hydrogen predominates during the early stages of the fermentation and carbon dioxide in the later stages.

Types.—Although *Cl. welchii* strains from gaseous gangrene form the same toxin, they are not immunologically homogeneous. Henderson[51] has observed that the Wilsdon types (see below) are homogeneous with respect to heat-stable antigen with the exception of Type D in which he found 6 kinds of antigens in 13 strains. Rodwell[52] has shown that a number of subtypes of each of the Wilsdon types may be distinguished by agglutination and precipitin tests and there are some cross reactions between types. As yet, however, serological identification of *Cl. welchii* is not practical. Four biochemical types have been suggested[53] on the basis of differences in the fermentation of glycerol and inulin, but these fermentative differences do not appear to be correlated with other variable characteristics.

Toxigenic bacilli closely resembling *Cl. welchii* and having immunologically related toxins have been isolated from lower animals. These are the lamb dysentery bacillus (*Bacillus agni*), a bacillus causing a disease of sheep called "struck" (*Bacillus paludis*), and a bacillus responsible for an enterotoxemia of sheep (*Bacillus ovitoxicus*). Wilsdon[54] has proposed that these be designated *Cl. welchii* but that four types be distinguished: *Cl. welchii* Type A—the gaseous-gangrene bacillus; *Cl. welchii* Type B—the lamb-dysentery bacillus; *Cl. welchii* Type C—the "struck" bacillus; and *Cl. welchii* Type D—the bacillus of enterotoxemia of sheep. Unless otherwise indicated, however, the name *Cl. welchii* always refers to the gas-gangrene bacillus, or Type A.

It has been shown that, as a group, these bacilli form seven immunologically distinct toxins and that the observed interrelationships of the toxins formed by the various types are attributable to the sharing of one or more of these components.[55] The effects produced by these toxins are as follows:

The α toxin is hemolytic, lethal to mice on intravenous injection, and produces necrosis on intradermal injection into guinea pigs and rabbits. Termed by Wilsdon the W factor and designated by some workers as the ζ toxin.

The β toxin is not hemolytic, mice injected intravenously develop spasmodic twitchings and die almost immediately, and it produces skin necrosis in guinea pigs and rabbits. Termed by Wilsdon the Z factor.

The γ toxin is not hemolytic, does not produce skin necrosis in guinea pigs, and is lethal to mice.

The δ toxin is hemolytic but is not lethal to mice and does not produce skin necrosis.

The ϵ toxin is not hemolytic, but produces skin necrosis in guinea pigs and rabbits and is lethal to mice. Termed by Wilsdon the X factor.

The θ toxin is hemolytic and lethal and probably produces necrosis in high concentrations. It is oxygen-labile and thermolabile and very similar in properties and immunological specificity, though not identical with, streptolysin O. It is the same as Prigge's toxin.

The η toxin has lethal activity only. It has been found in only one strain (Lechien) of Type A as yet examined and its occurrence or absence in the other types is not definitely established.

[51] Henderson: Jour. Hyg., 1940, *40*:501.
[52] Rodwell: Australian Vet. Jour., 1941, *17*:58.
[53] Simonds: Jour. Inf. Dis., 1915, *16*:31, 35; Henry: Jour. Path. Bact., 1917, *21*:344.
[54] Wilsdon: Inst. Animal Path., Cambridge; 2nd report of the Director, 1931, p. 53.
[55] See the review by Oakley: Bull. Hyg., 1943, *18*:781.

The optimal conditions of pH, incubation time and composition of the medium differ from one toxin to another, and a type producing more than one toxin will produce only those for which the conditions are optimal.[56] The distribution among the Wilsdon types of the ability to form these toxins is indicated in the accompanying table. The terminology of the toxins has been somewhat confused owing to differences in those used by various workers; that used here has been generally agreed upon.[57]

Of these the α toxin has been of greatest interest. It was observed independently by Nagler[58] and by Seiffert[59] that an opalescence is produced in human serum by the addition of filtrate containing the α toxin. This is known as the *Nagler reaction.* On the assumption that the opalescence is a result of splitting lipoprotein, it was shown by Macfarlane, Oakley and Anderson[60] that incubation of such filtrate with a saline extract of egg yolk, "lecithovitellin," resulted in the separation of a curd of fat. It was then shown by Macfarlane and Knight[61] that an enzymatic splitting of free lecithin into phosphocholine and stearyloleylglyceride occurs, and the toxin

TOXINS FORMED BY THE WILSDON TYPES OF CL. WELCHII

Type	Toxins						
	α	β	γ	δ	ϵ	θ	η
Cl. welchii Type A (bacillus of gas gangrene)	+	−	−	−	−	+	+
Cl. welchii Type B (*Bacillus agni*)	+	+	+	±	−	+?	?
Cl. welchii Type C (*Bacillus paludis*)	+	+	+	+	−	+?	?
Cl. welchii Type D (*Bacillus ovitoxicus*)	+	−	−	−	+	+?	?

appears to be a lecithinase. Since phosphocholine is water soluble, it has been suggested that the α toxin may be estimated by measurement of the liberation of soluble phosphorus under standard conditions.

There appears to be no simple method of typing *Cl. welchii* and the presence or absence of the various toxins must be demonstrated. The differentiation of the toxins suggested by Wilsdon[54] is relatively simple but too crude for most purposes. A precise method of determining the toxic components of a filtrate using monospecific antisera is outlined by Oakley.[55]

Pathogenicity for Man.—*Cl. welchii* is, perhaps, the most important cause of gaseous gangrene and is found either alone or mixed with other anaerobes in the majority of cases of this disease. Tissue injury is a usual,

[56] Stewart: Pub. Health Repts., 1940, *55*:753–775.
[57] *Cf.* Nature, 1942, p. 56.
[58] Nagler: Brit. Jour. Exp. Path., 1939, *20*:473.
[59] Seiffert: Ztschr. f. Immunitätsf., 1939, *96*:515.
[60] Macfarlane, Oakley and Anderson: Jour. Path. Bact., 1941, *52*:99.
[61] Macfarlane and Knight: Biochem. Jour., 1941, *35*:882.

perhaps essential, preliminary to infection, but once the bacilli are established they invade the surrounding tissue rapidly. They apparently travel along the interstitial tissue of the muscle and are often found beyond the gangrenous area. The large amount of hyaluronidase produced undoubtedly facilitates the spread of the infection.

Although most commonly found in gangrene, *Cl. welchii* has also been observed in closed abscesses in uterine infections, and in infections of the gastro-intestinal, genito-urinary, and biliary tracts. It has been isolated from the blood during life, but septicemia in man is much less common than in experimental animals, although blood invasion occurs frequently in man during the agonal period or immediately following death. Study of the "foamy organs" sometimes observed at autopsy has shown that the presence of gas in the internal organs shortly after death is often attributable to an invasion by this microorganism.

Cl. welchii is a normal inhabitant of the human intestine and is constantly present in small numbers; in fact, it has been used to a certain extent in Europe as an indicator of fecal pollution of water (p. 223). The toxemia of acute intestinal obstruction has been attributed by some to the proliferation of *Cl. welchii* in the bowel followed by absorption of formed toxin, but it is now clear that such a relationship does not exist. This bacterium is, however, found with some frequency in gangrenous appendicitis and it has been reported[62] that antitoxin is of value in the treatment of perforative appendicitis.

Since infection with *Cl. welchii* is frequently characterized by gross blood destruction, jaundice and anemia, a possible relationship between this bacterium and various anemias has been of some interest. A pernicious and fatal anemia may be produced in experimental animals by intratibial inoculation of culture[63] or a temporary but severe anemia by inoculation of filtrate. Both natural and experimental infections, therefore, lead to the development of a severe anemia which is probably due to the continuous release of the hemolytic toxin.

Pathogenicity for Animals.—Natural infections in lower animals with *Cl. welchii* types have been referred to above. The occurrence of the gas-gangrene bacillus, however, is rare; local abscesses have been observed in dogs and rabbits following injury. Experimentally certain strains are pathogenic for guinea pigs, pigeons and mice, less so for rabbits. If a rabbit or guinea pig is killed a few minutes after intravenous injection of *Cl. welchii* and the body incubated at 37° C., gas is produced in a few hours through the body and the phenomenon of "foamy liver" reproduced. This phenomenon is not strictly specific for *Cl. welchii;* it may be produced by similar inoculations with several other anaerobes, though the results are less striking.

The classic *welchii* toxin, the α toxin, is not a powerful one; the MLD for a mouse is usually about 0.25 cc. of liquid culture. Antitoxins may be produced, however, which have both prophylactic and therapeutic value,

[62] Bower, Mengel and Paxon: Jour. Immunol., 1938, *34:*185.

[63] Torrey and Kahn: Amer. Jour. Path., 1928, *4:*621; Fellinger and Pfleger: Ztschr. f. gesamt. exp. Med., 1935, *96:*121.

and *welchii* is included in polyvalent antitoxic sera for gas gangrene. Antitoxin to the α toxin appears to be far more important than that to the θ toxin.[64] It may be noted that it is difficult to develop agglutinins for *Cl. welchii* and an antibacterial immunity does not protect against infection.

3. Clostridium Novyi (Clostridium Oedematiens)

The third important anaerobe in gaseous gangrene was probably first discovered by Novy[65] in 1894 in a study of "malignant edema" in guinea pigs, and was designated "*Bacillus oedematis maligni Nr. II.*" It was named *Bacillus novyi* by Migula in 1900. In 1915 Weinberg and Séguin isolated several strains of this bacillus, but first regarded it as a new species and named it *Bacillus oedematiens*. The French name has been used by European workers although the bacillus is properly known as *Clostridium novyi*.

Fig. 122.—*Clostridium novyi* from pure culture. The slight tendency to curvature is apparent in some of the vegetative cells. Note the subterminal spores and the absence of free spores in large numbers. Fuchsin; × 1050.

Cl. novyi is noteworthy not only for its importance in gaseous gangrene, but also because of its strong, soluble exotoxin, which compares in potency with the toxin of the diphtheria and tetanus bacilli, and for which an equally powerful antitoxin can be produced.

Morphology.—Novy's bacillus is a large, relatively thick rod, 2.5 to 10 μ in length and 0.8 to 1 μ in breadth, and occurs singly and in chains. In cultures navicular and curved forms are found; in animal transudates the shorter form predominates. Its numerous spiral flagella, which often become tangled in "bouquets," have been emphasized in nearly all the published descriptions. The rod is non-motile under ordinary conditions of examination, for movement is markedly inhibited in the presence of air. Subterminal spores are produced but sparsely as a rule and best in non-fermentable media. The bacillus is gram-positive.

[64] Evans: Brit. Jour. Exp. Path., 1943, *24*:81.
[65] Novy: Ztschr. f. Hyg., 1894, *17*:209.

Young colonies in deep dextrose agar have a yellowish, opaque, irregular center surrounded by a delicate corona of short filaments. Later the colony clears, the center becomes cloudy, and is surrounded in forty-eight hours with a corona of tangled filaments. Surface colonies are extremely delicate, flattened, transparent, bluish gray, with irregular contours, and there is slight hemolysis on blood agar.

Physiology.—*Cl. novyi* is a strict anaerobe, and grows well at 37° C. in ordinary media and especially abundantly in the presence of a fermentable sugar. Meat and brain are not darkened; the former may be turned slightly pink or bleached. Gelatin is liquefied, but coagulated serum and egg are not digested. McLeod and Gordon[66] have reported that *Cl. novyi* may be distinguished from the other sporulating anaerobes by culture on blood agar containing benzidine; the colonies are blackened due to the presence of hydrogen peroxide. Nitrates are not reduced and indol is not formed, but hydrogen sulfide is produced. Acid is produced very slowly in litmus milk; after ten to thirty days' incubation a fine flocculent clot appears which is not digested. Dextrose is fermented but lactose is not; the latter property serves to differentiate *Cl. novyi* from *Cl. septicum* and *Cl. chauvei*. There is not complete agreement as to the other fermentation reactions.

Pathogenicity for Man.—The relatively frequent occurrence of *Cl. novyi* in gas gangrene has been noted earlier. The disease is characteristically a toxemia, although septicemia is not rare. Like *Cl. welchii*, Novy's bacillus is often a terminal invader. In pure infections there is less tissue destruction than with *Cl. welchii* or *Cl. septicum*. The postmortem findings consist mainly in a massive localized edema, with neither the extensive gas production of the former nor the sanguineous necrosis of the latter.

Pathogenicity for Animals.—Natural infections due to *Cl. novyi* have been observed in guinea pigs, cattle, horses and hogs. Guinea pigs, rabbits, rats, mice, cats, sheep, horses and pigeons are susceptible to small doses of culture. Subcutaneous, intramuscular and intravenous inoculations reproduce the disease experimentally. Toxicity and pathogenicity are easily lost, however; Novy's original strain, which still survives in several laboratories, has long since failed to kill experimental animals. This is true also of strains isolated within five to ten years.

The action of the toxin freed from bacteria is very similar to that of whole cultures. Sublethal, subcutaneous doses of toxin or culture produce a peculiar non-hemorrhagic, gelatinous local edema which reaches its maximum in two or three days. It may be followed by small, superficial hemorrhages, after which it is slowly absorbed, leaving a slightly sclerotic scar. Such lesions appear not to form open phlegmons, as in the case of *Cl. welchii* cultures, and may also be contrasted with those of the vibrion septique and *Cl. chauvei*, which, if they appear at all, are always fatal. Washed cultures are harmless.

Antitoxin has been produced in rabbits, sheep and horses by successively increased doses of toxic filtrates. The antitoxin has prophylactic and, to some extent, therapeutic value under experimental conditions, and is now represented in several polyvalent American sera for anaerobic infections.

[66] McLeod and Gordon: Jour. Path. Bact., 1940, *50*:167.

Agglutinating antisera have been produced in rabbits, but are active for homologous strains only;[67] it may be noted that spontaneous agglutination frequently occurs with this microorganism.

4. Clostridium Histolyticum

Among the new species of bacteria discovered by Weinberg and Séguin in war wounds, none is of more interest than *Cl. histolyticum*, so named because of its remarkable liquefying action upon living tissues. It may be somewhat more common in gaseous gangrene than indicated earlier. It has also been recovered from soil and human feces in California,[68] and from 5 out of 6 African poisoned arrows examined.[69]

Morphology.—*Cl. histolyticum* is a gram-positive motile rod, 3 to 5 μ long and 0.5 to 0.7 μ wide, that forms subterminal clostridial spores. In smears from lesions it appears generally in the form of single or paired short

Fig. 123.—*Clostridium histolyticum* from pure culture. Note the characteristic short rods with rounded ends and the clostridial subterminal spores. Fuchsin; × 1050.

rods with rounded ends. The flagella, often more than 20 in number, are peritrichal. Deep agar colonies vary, according to the consistency of the medium, from compact lobulate globules in 2 per cent agar to fluffy semi-transparent or even cottony balls in lower concentrations. Surface colonies are minute, round dewdrops and are hemolytic on blood agar.

Physiology.—Originally described as an obligate anaerobe, *Cl. histolyticum* is capable of a delicate transparent growth upon the surface of meat infusion agar and is perhaps best regarded as microaerophilic or as a facultative anaerobe.

This bacillus is actively proteolytic; not only is gelatin liquefied but meat and brain and coagulated serum and egg are digested. In older cultures a precipitate of tyrosine crystals appears. Nitrates are not reduced and indol

[67] See, however, Turner and Eales: Australian Jour. Exp. Biol. Med. Sci., 1943, *21*:79.
[68] Peterson and Hall: Proc. Soc. Exp. Biol. Med., 1923, *20*:502; Hall: *ibid.*, 1923, *21*:198.
[69] Hall and Whitehead: Jour. Inf. Dis., 1927, *41*:51.

is not formed. No carbohydrates are known to be fermented in spite of statements to the contrary regarding dextrose. The action on milk is slow, but after several days a soft clot is usually formed and then slowly digested. *Cl. histolyticum* is, clearly, a proteolytic type.

Pathogenicity.—Infection with *Cl. histolyticum* alone is probably a rare occurrence; mixed cultures with other anaerobes and aerobes appear to be the rule both in war wounds and in infections observed in horses. Most pure cultures of this bacillus are pathogenic under experimental conditions for rabbits, guinea pigs, mice and rats, but there is considerable difference between strains. Subcutaneous inoculation of 1 or 2 cc. of a twenty-four-hour broth culture generally produces a local tumefaction followed in twenty-four to forty-eight hours by complete sloughing of the overlying skin; then, as a rule, healing slowly occurs. Intramuscular inoculation causes swelling, followed by progressive myolysis. If the gluteus muscle of a guinea pig is selected for the inoculation, it may be entirely denuded from the bone within twenty-four to forty-eight hours. The tissues literally drip away, and in some cases the limb may be disarticulated. Curiously there is often little or no intoxication of the animal, but death usually follows through peritonitis due to perforation of the peritoneum. There is occasionally invasion of the blood stream, but generally septicemia does not occur. There is never any gas formation in such pure infections.

Bacteria-free filtrates have a lytic action which can be demonstrated if sufficiently large quantities (5 cc.) are injected. The most characteristic effect is the formation of a sterile hematoma filled with uncoagulated blood in which the red corpuscles are still intact. According to Pasternack and Bengtson[70] deep intramuscular inoculation produces an edema which disorganizes and separates the tissue, while gross lesions are observed only occasionally following intravenous inoculation. Agglutinating and antitoxic sera have been produced.

5. Clostridium Sporogenes

Clostridium sporogenes, which in pure culture is a harmless saprophyte, is included here because it is frequently associated with the pathogenic anaerobes in mixed gangrenous infections, very possibly because it is so widely distributed in nature. It has frequently been confused with the pathogenic forms; not only have cultures labeled something else proved to be *sporogenes*, but there has been a tendency to regard "atoxic variants" or "atoxic strains" of pathogens as *Cl. sporogenes*. On the other hand, the presence of "atoxic variants" of pathogenic species is in many cases attributable to mixed cultures containing *sporogenes*. The spores of this microorganism are unusually hardy and invariably survive with those of the pathogens or even after the pathogenic spore-formers are killed in preliminary selective heating.

Morphology.—*Cl. sporogenes* is an actively motile, gram-positive, slender rod 3 to 7 μ in length and 0.6 to 0.8 μ in breadth, with rounded ends. The cells occur individually, in pairs, in short chains, and sometimes in filaments. The spores are oval, eccentric to subterminal, and

[70] Pasternack and Bengtson: Pub. Health Repts., 1940, *55*:775.

swell the vegetative cell. The flagella are peritrichal. The deep agar colonies have the appearance of woolly balls with a dense, compact center. Surface colonies on blood agar are hemolytic, transparent and usually rhizoid, or ameboid, with a slightly raised center; they appear moist and at first may resemble minute dewdrops.

Cl. sporogenes requires strictly anaerobic conditions for growth and will grow upon all the ordinary media. It has been cultivated in synthetic solutions containing tryptophane, leucine, tyrosine, arginine and phenylalanine, together with an unknown substance termed "sporogenes vitamin."[71] Its optimum temperature is 37° C. but it will grow at temperatures as high as 50° C.

This bacillus is actively proteolytic; it produces blackening and digestion of brain and meat media, coagulated egg and serum. An excess of fermentable sugar delays or inhibits this proteolysis and the presence of

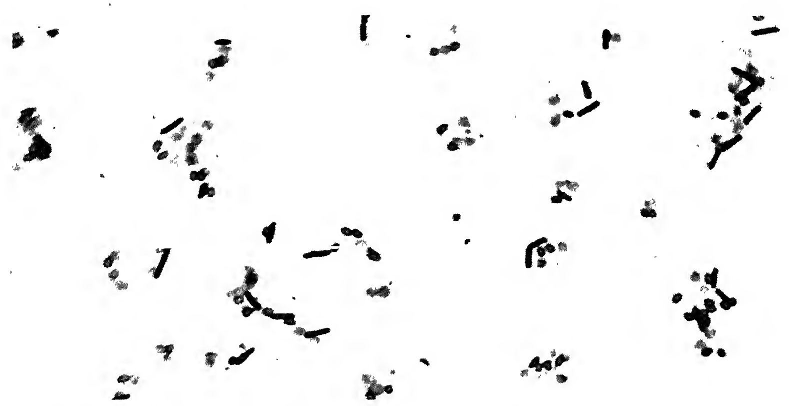


Fig. 124.—*Clostridium sporogenes* from pure culture. Note the close morphological resemblance of this species to the pathogenic forms. Fuchsin; × 1050.

metallic iron or certain iron salts accelerates it. Tyrosine crystals are not obvious. Gelatin is liquefied and blackened, hydrogen sulfide is produced, but indol production is doubtful and nitrates are not reduced to nitrites, Reports on sugar fermentations are conflicting. According to Bergey (1939). acid and gas are formed from dextrose, levulose, galactose and maltose, while lactose, sucrose, salicin and inulin are not fermented. Growth on milk is at first slow; in forty-eight to seventy-two hours a clot is formed and progressive liquefaction occurs with abundant gas and acid formation until the casein is completely peptonized.

Pathogenicity.—There is no authentic record of a natural infection attributable to *Cl. sporogenes* alone. It has been claimed that this bacillus is a factor in certain intestinal disorders, but its frequent occurrence in the intestinal tract of healthy men and animals is not in accord with this supposition.

[71] Fildes and Richardson: Brit. Jour. Exp. Path., 1935, *16*:326.

In animal experiments relatively large doses are required to produce lesions; less than 5 cc. of a young dextrose broth culture injected subcutaneously in guinea pigs, which are the most susceptible experimental animals, usually results in only a local manifestation. In a few hours the immediately overlying hair loosens, the skin becomes gangrenous and raised slightly over an area of subcutaneous tissue digestion in which a small amount of gas appears. Such animals usually show no systemic involvement and the lesion heals in a few days, leaving a necrotic scar which heals slowly. The reaction to intramuscular injection is only slightly more severe.

The most obviously important of the pathogenic manifestations of *Cl. sporogenes* (and probably of other putrefactive anaerobes) is that of a mutual acceleration in metabolism which occurs during growth with the more definitely pathogenic anaerobes, especially *Cl. welchii*, *Cl. septicum* and *Cl. novyi*. While the presence of various aerobes is in some degree stimulating to the growth of obligate anaerobes (due partly, as Pasteur suggested, to the absorption of oxygen, but also to other unknown factors) the presence of putrefactive anaerobes greatly enhances the pathogenicity of the non-putrefactive pathogens. The proteolytic forms supply protein-split products which the fermentative types are unable to elaborate so rapidly. It may be noted that, while an admixture of a proteolytic culture reduces the minimum fatal dose of a non-proteolytic anaerobe experimentally, the admixture of *sporogenes* filtrate with toxic filtrates of *Cl. welchii* or *Cl. novyi* has no such action; there may, in fact, be a diminution of toxicity.

BLACKLEG (CLOSTRIDIUM CHAUVEI)

Blackleg, also known as quarter evil and symptomatic anthrax (not to be confused with anthrax, Chapter 27), is an important, widespread, acute disease affecting cattle. It occurs wherever cattle are kept and is prevalent throughout the United States with the possible exception of the Southern Atlantic and Eastern Gulf States. The name blackleg, like gaseous gangrene, has been applied to affections due to various anaerobes; in some instances *Cl. septicum* or, rarely, *Cl. novyi* is found, but the principal cause is *Cl. chauvei*. Just as *Cl. welchii* (Type A) is not involved in natural infections of lower animals, so *Cl. chauvei* has never been shown to be responsible for any human infection.

Although the bacilli had been earlier observed and the disease transmitted by the injection of the serous fluid from an infected animal into a healthy animal, *Cl. chauvei* was cultivated and its causal relation to blackleg established by Arloing, Cornevin and Thomas[72] in 1887.

Morphology.—*Cl. chauvei* is a gram-positive, motile, sporulating rod. The size is variable, ranging from 3 to 8 μ in length and about 1 μ in breadth. The cells occur singly as a rule; in contrast with *Cl. septicum*, there is little tendency to form chains or filaments. The spores are subterminal and oval, swelling the vegetative cell in which they occur. Sporulation is often preceded by a marked swelling of the vegetative cell.

[72] Arloing, Cornevin and Thomas: *Le Charbon Symptomatique du Boeuf*. Paris. 1887.

Deep agar colonies are minute, compact and downy. On the surface of blood agar well separated colonies are flat, round or leaf-like, and hemolytic.

Physiology.—This bacillus is a strict anaerobe and, like *Cl. sporogenes*, grows at temperatures as high as 50° C., though the optimum is 37° C. It will grow on the usual laboratory media but is best cultivated in meat or brain medium. These are never discolored nor digested by pure cultures, but they may be slightly softened. Gelatin is liquefied, but coagulated serum and egg are not. Hydrogen sulfide is produced, but indol is not formed and nitrates are not reduced to nitrites. Dextrose, levulose, galactose, maltose, sucrose and lactose are fermented with the formation of acid and gas; inulin, salicin, mannitol, dulcitol and glycerol are not fermented. It may be noted again that this microorganism may be differentiated from the closely related *Cl. septicum* on the basis of the sucrose and salicin fermentation. Litmus-milk cultures become acid and the casein is precipitated. but peptonization does not occur.

Fig. 125.—*Clostridium chauvei* from pure culture. Subterminal oval spores are apparent; note the swollen pre-sporulating cell on the far right. Fuchsin; × 1050.

Pathogenicity.—Natural infections due to *Cl. chauvei* occur principally in cattle. There are still several obscure features in the epidemiology. The disease occurs at special seasons of the year, is connected with certain localities, and is said to show a distinct predilection for the best young stock. The portal of entry is uncertain; whether it enters by way of minute abrasions on the skin or through the gastro-intestinal mucosa is quite unknown. Experimentally, *Cl. chauvei* is pathogenic for cattle, sheep, goats, guinea pigs and mice; horses, asses, hogs, rabbits, rats and pigeons are somewhat refractory.

The symptoms in animals consist in crepitant localized swellings, which in natural infections occur on the thighs, neck or shoulders. The animals become stupid, feverish and anorectic. Treatment is rarely successful, and sick animals usually die in one to two days. The mechanism of the disease is that of progressive bacteremia. A weak exotoxin is produced.

Immunization.—A method of prophylactic inoculation which was devised by Arloing, Cornevin and Thomas[72] has been widely used in active immunization. The Lyon[73] vaccine, as distributed by the Bureau of Animal Industry of the United States Department of Agriculture,[74] is prepared as follows: The muscle tissue from a fresh blackleg tumor is pulverized in a mortar, extracted with water, and the extract dried at 35° C. The dry brown scale which results is suspended in water (2 parts), heated for six hours at 95° to 99° C., and injected in appropriate quantities as determined by test and specified on each package distributed. The dried material retains a high degree of activity for several years. Some commercial firms distribute this vaccine in the form of strings to be sewn into the flesh; others dispense the powdered vaccine in the form of pellets which are inoculated by means of a "pill gun." In 1901–2 (July 1 to June 30) 565,628 cattle were vaccinated in the United States. During the previous season 14,817 deaths had occurred; in a similar period after vaccination the number of deaths was only 2902. It should be noted that some of the commercial vaccines have been found to be seriously contaminated and lacking in protective power.[75]

Bacteria-free filtrates of edema fluid expressed from the flesh of guinea pigs dead of blackleg or of liquid cultures have been used as immunizing agents with promising results.[76]

Agglutinating antisera may be prepared against *Cl. chauvei*, and this species appears to be immunologically homogeneous.

CLOSTRIDIUM BOTULINUM

The type of food poisoning termed botulism has been discussed elsewhere (p. 241) and need not be considered further here. In Germany botulism was first definitely observed in 1785 and was and is associated, though by no means exclusively, with the consumption of sausages; hence the not altogether appropriate name botulism (Lat., *botulus*, sausage). The causative bacterium was isolated by van Ermengem[77] in 1896 and named *Bacillus botulinus*. It is now known as *Clostridium botulinum*.

Morphology.—*Cl. botulinum* is a large, pleomorphic, gram-positive, motile, sporulating rod, 4 to 6 μ in length and 0.9 to 1.2 μ in breadth. The cells occur singly, in pairs and in chains. There are 4 to 8 peritrichal flagella. The spores are subterminal and oval and distend the vegetative cells containing them. Spore formation is variable from strain to strain, some strains producing spores abundantly, others sparsely; but in general spore formation is best in sugar-free media.

Deep agar colonies are translucent, globular and diffuse, or flat and heart-shaped or disk-shaped, according to the consistency of the medium. Surface colonies are relatively large, 5 to 10 mm. in diameter, glistening,

[73] Referring to Lyons, France.

[74] *Cf.* Norgaard: U. S. Dept. Agr., Bur. Animal Ind., Circular No. 31, 1907.

[75] Franklin and Haslam: Jour. Inf. Dis., 1916, *19*:408; *ibid.*, 1920, *26*:424.

[76] *Cf.* Goss: Kans. Agr. Exp. Station Circular, 1919, 75, p. 4. The preparation of culture filtrates is considered in detail by Kelser: Jour. Agr. Res., 1918, *14*:253.

[77] van Ermengem: Centralbl. f. Bakt., 1896, *19*:442; Ztschr. f. Hyg., 1897, *26*:1.

translucent at the edges with a thicker brownish center, filamentous, and hemolytic on blood agar.

Physiology.—*Cl. botulinum* may be grown on the usual laboratory media under strict anaerobic conditions; cultivation on synthetic solutions has indicated that the amino acids cystine, leucine, lysine, glycine and proline are required.[78] Amino acids are decomposed by coupled oxidation-reduction reactions rather than by direct oxidation.[79] Brain, meat and coagulated protein media are blackened and digested, gelatin is liquefied. Milk is peptonized. Hydrogen sulfide is produced, but nitrates are not reduced to nitrites and indol is not formed. Dextrose, levulose and maltose are fermented; the fermentation of other sugars is variable from strain to strain and type to type. The spores are highly resistant and withstand boiling for thirty minutes to twenty-two hours, and autoclaving at 120° C. for as long as twenty minutes.

Fig. 126.—*Clostridium botulinum* Type A from pure culture. Note the subterminal swollen spores and free unstained spores admixed with the vegetative cells. Fuchsin; × 1050.

Irrespective of the presence of fermentable sugar, a potent soluble toxin is produced which resembles other soluble toxins in most respects. It is, however, unusually stable to heat; heating to 80° C. for thirty minutes or boiling for ten minutes is required to destroy it. Botulinum toxin is unique in that it is not destroyed by the digestive enzymes of the gastrointestinal tract and hence is effective when given by mouth. It is the most potent bacterial toxin known; the guinea-pig MLD may be as small as 1×10^{-6} cc. of broth culture.

Types.—*Cl. botulinum* is subdivided into a number of types which differ from one another in that their toxins are immunologically different. The best known of these are Types A and B, which, in the past, have been regarded as solely responsible for human botulism. It is supposed that van Ermengem's original culture, now no longer available, was Type B.

[78] Burrows: Jour. Inf. Dis., 1933, *52*:126.
[79] Clifton: Jour. Bact., 1940, *39*:485.

Additional types have been described since the early 1920's. A toxin-producing anaerobic bacillus isolated from fly larvae (the ingestion of which was associated with a paralytic disease of chickens) has been designated Type C.[80] A closely related bacillus was isolated by Seddon[81] from botulism of cattle in Australia which he designated *Bacillus parabotulinus.* This bacillus is now designated *Cl. botulinum* Type Cβ and the fly larvae bacillus *Cl. botulinum* Type Cα. The toxins of these subtypes of Type C are related in that Cα antitoxin protects against both Cα and Cβ toxin, but Cβ antitoxin protects against Cβ toxin but not Cα toxin.

A South African strain first described by Theiler and Robinson[82] designated by them *Clostridium parabotulinum equi,* has been further studied by Meyer and Gunnison[83] and given the name *Clostridium botulinum* Type D, for its toxin is not neutralized by the antitoxins of Types A, B or C. Still another type, *Clostridium botulinum* Type E, has been isolated[84] in Russia from fish consumption of which resulted in human botulism. The Type E toxin is not neutralized by antitoxins of the other four types. There appear to be some strain differences in the action of Type E toxins though complete cross neutralization occurs.[85] Type E, it may be noted, has been found as a cause of human botulism in the United States.[86]

At present, then, there are 5 types of *Clostridium botulinum* which differ from one another in the immunological specificity of their toxins. These types are immunologically heterogeneous, as indicated by agglutination tests, and are not biochemically or culturally distinguishable. As a group, however, they may be divided into two biochemical types, the one proteolytic (sometimes designated ovolytic but digesting proteins other than coagulated egg albumin), whose cultural characteristics have been described above, and the other saccharolytic or fermentative in character, whose members do not hydrolyze coagulated native proteins. The proteolytic group includes Type A and some strains of Type B (the majority of the American Type B strains are proteolytic, while a great many of the European Type B strains are not). The non-proteolytic group includes some strains of Type B, and, so far as is known, all strains of Types C, D and E. Bengtson[87] has suggested that only the non-proteolytic varieties be designated *Cl. botulinum* and that the proteolytic varieties be termed *Cl. parabotulinum* (because van Ermengem's original strain was non-proteolytic)—a suggestion that has been adopted in the Bergey classification. It would appear undesirable, however, to designate practically all of the bacilli causing human botulism in the United States as "parabotulinum."

[80] Bengtson: Pub. Health Repts., 1923, *38*:340.
[81] Seddon: Jour. Comp. Path. and Therap., 1922, *35*:147, 275.
[82] Theiler and Robinson: Rev. Gen. de Med. Vet., 1927, *36*:193.
[83] Meyer and Gunnison: Jour. Inf. Dis., 1929, *45*:106.
[84] *Cf.* Gunnison, Cummings and Meyer: Proc. Soc. Exp. Biol. Med., 1936, *35*:278; Kushnir, Brun and Paikina: Ztschr. Microbiol. Epidemiol. Immunitätsf. (U.S.S.R.), 1937, *19*:80 (in English 85).
[85] Hazen: Proc. Soc. Exp. Biol. Med., 1942, *50*:112.
[86] Hazen: Science, 1938, *87*:413; Geiger: Jour. Amer. Med. Assn., 1941, *117*:22.
[87] Bengtson: U. S. Pub. Health Ser. Hyg. Lab. Bull. No. 136, 1924.

Pathogenicity for Man.—Human botulism is invariably the result of eating preserved foods in which the bacillus has grown and produced toxin. In Europe most cases have been due to the consumption of various kinds of preserved meats, such as sausage, ham, potted goose or duck and the like, while in the United States the incriminated foods have been canned vegetables for the most part. There are surprisingly few cases of botulism in this country in view of the ubiquitous distribution of *Cl. botulinum* spores in the soil—Type A has been found most commonly in the Rocky Mountain and Pacific Coast states, while Type B predominates in the Mississippi Valley, Great Lakes region, and Atlantic Coast states.[88] Type A predominates in English soils, though Type B may be found also.[89] From 1899 to 1935 there were 261 reported cases of botulism in the United States,[90] the greatest numbers in any one year being 23 in 1922 and 22 in 1935. Over that period of time 101 cases were reported from California, by far the greatest number in any single state.

TYPES OF CLOSTRIDIUM BOTULINUM

Type	Synonym	Biochemical Character	Disease	Antitoxin
A		Proteolytic	Botulism of man, limberneck of chickens	Specific
B		Some strains proteolytic	Botulism of man, limberneck of chickens	Specific
Cα	Fly larvae bacillus	Non-proteolytic	Paralytic disease of chickens, botulism of wild ducks	Neutralizes Cβ toxin
Cβ	*Cl. parabotulinum*	Non-proteolytic	Forage poisoning of cattle (Australia)	Specific
D	*Cl. parabotulinum equi*	Non-proteolytic	Lamziekte of cattle (Africa)	Specific
E		Non-proteolytic	Botulism of man	Specific

As in the case of the other sporulating anaerobes, the disease produced by *Cl. botulinum* is an intoxication; in botulism, in fact, there is no invasion of the tissues and the toxin is preformed outside the body. Even under experimental conditions in which massive doses of spores have been injected, it is probable that no infection has been set up.

Until the recent reports of the occurrence of Type E, Types A and B were the only types of *Cl. botulinum* involved in human botulism. The pharmacological activities of these toxins are substantially identical; the effect appears, in the main, to be exerted on the peripheral nerve endings, possibly those of the autonomic system, and paralysis of the motor nerve-

[88] Meyer and Dubovsky: Jour. Inf. Dis., 1922, *31*:559.
[89] Haines: Jour. Hyg., 1942, *42*:323.
[90] Meyer: Calif. and Western Med., 1936, *44*:No. 5.

end plates in the striated muscles and the diaphragm results. Whether there are pathological changes in the nerve cells of the central nervous system, such as degeneration of the Nissl bodies, is uncertain. The symptoms include vomiting, constipation, ocular paresis and pharyngeal paralysis. Death may occur within a day of the onset of symptoms or may be delayed for as long as a week. At autopsy the liver, kidneys and meninges are congested and there may be thrombosis. The case fatality is variable; in the United States it has been 60 to 70 per cent, but in Germany it is much lower, perhaps 25 per cent.

Pathogenicity for Lower Animals.—Associated with human cases of botulism there have been numerous outbreaks of limberneck, a paralytic disease, among fowls fed the toxin-containing food. Other forms of botulism in lower animals occur under natural conditions, however. *Cl. botulinum* Types C and D appear to be associated exclusively with the disease in lower animals. Certain forms of forage poisoning in cattle and horses in Australia are botulism,[81] but whether the bacilli grow and form toxin in the fodder or whether the disease results from the ingestion of rabbit carrion is not entirely clear. The South African disease of cattle, lamziekte,[82] is botulism resulting from the ingestion of contaminated carrion. In the United States botulism of wild ducks and other waterfowl due to Type Cα is prevalent and causes the death of thousands of ducks each year.[91] The source of the toxin ingested by these fowl is uncertain.

Experimentally rabbits, guinea pigs, mice, monkeys, cats and dogs are susceptible to toxin administered parenterally or *per os*. The symptoms are similar to those of naturally infected animals and of man, and the postmortem findings are much the same. Experimental animals vary widely in their susceptibility to the toxins of the various types of *Cl. botulinum.*

Immunity.—Botulinum toxin is an excellent antigen and high-titer antitoxic sera may be produced. Under experimental conditions these antitoxins have marked prophylactic value, but their therapeutic efficacy is slight. It may be pointed out that in botulism, as in tetanus, the symptoms are a consequence of the injury to the nerve tissue and the administration of antitoxin serves only to neutralize circulating toxin. The almost complete lack of therapeutic effect of botulinum antitoxin in human botulism is undoubtedly attributable to the inevitable too-late administration.

Animals may be actively immunized against botulism by the inoculation of formol toxoid. In Australia botulism of sheep and cattle has assumed sufficient proportions to justify such active immunization, and it has been applied on a small scale.[92]

DIFFERENTIATION OF THE SPORULATING ANAEROBES

In many respects the isolation and identification of the sporulating anaerobic bacilli is somewhat more difficult than in the case of the aerobic and facultatively anaerobic bacteria. Primary cultures may be inoculated into deep brain medium and, after incubation, examined for spores, heated

[91] *Cf.* Gunnison and Coleman: Jour. Inf. Dis., 1932, *51*:542. See also Kalmbach: U. S. Dept. Agr., Bur. Biol. Surv. Wildlife Res. and Manag. Leafl. BS-120. 1938.

[92] *Cf.* Bennetts and Hall: Australian Vet. Jour., 1938, *14*:105.

to 80° C. for ten minutes, then subcultured. Representative colonies may be picked from shake cultures or from the surface of plates incubated either in an anaerobic jar or in the Spray dish. A procedure for the rapid identification of the anaerobes associated with gaseous gangrene has been given in some detail by Reed and Orr.[93]

Spray[94] has divided these bacilli into main groups on the basis of reaction in iron milk[95] and has developed a key for their further differentiation and identification on the basis of morphology, physiology and pathogenicity. Of no small practical utility, his key is outlined below in an abridged form; the original paper should be consulted for details concerning other anaerobic species and the preparation of the media.

SPRAY'S KEY TO THE SPORULATING ANAEROBES

I. Iron milk: active gaseous fermentation, early coagulation (12–48 hours), no digestion of clot, no blackening.
  Lead acetate: strongly blackened.
  Nitrite +, indol −, gelatin +, motility −.
  Glucose +, lactose +, sucrose +, salicin −.
  Spores ovoid, central-excentric, not swelling rod, infrequently observed and not in fermentable sugars.
  Clostridium welchii

II. Iron milk: inactive gaseous fermentation, late coagulation at 4–6 days, no digestion of clot, no blackening.
  Lead acetate: no blackening, no browning.
  Nitrite +, indol −, gelatin +, motility +.
  Glucose +, lactose +, sucrose −, salicin +.
  Spores ovoid, abundant, excentric-subterminal, swelling rod.
  Clostridium septicum

III. Iron milk: inactive gaseous fermentation (long continued), late, if any, coagulation (10–20–30 days or not even at 60 days), no digestion, no blackening.
  A. Lead acetate: strongly blackened.
    1. Lactose −.
      Nitrite −, indol −, gelatin +, motility +.
      Glucose +, sucrose −, salicin −.
      Spores ovoid, not abundant, excentric-subterminal, swelling rod.
      Clostridium novyi
    2. Lactose +.
      Nitrite +, indol −, gelatin +, motility +.
      Glucose +, sucrose +, salicin −.
      Spores ovoid, abundant, excentric-subterminal, swelling rod.
      Clostridium chauvei
  B. Lead acetate: no blackening, no browning.
    Nitrite −, indol −, gelatin +, motility +.
    Glucose +, lactose −, sucrose −, salicin −.
    Spores ovoid, not abundant, terminal, slightly swelling rod.
    Clostridium botulinum C

[93] Reed and Orr: Proc. Soc. Exp. Biol. Med., 1941, *48*:535; War Med., 1941, *1*:493.
[94] Spray: Jour. Bact., 1936, *32*:135.
[95] Fresh whole milk is sterilized in deep tubes, each of which contains a 50 × 7 mm. piece of No. 26 gauge black stove-pipe iron.

IV. Iron milk: inactive gaseous fermentation, more or less rapid digestion (with or without previous clotting), strongly blackened early (48 hours) or late (8–9 days).

A. Lead acetate: strongly and rapidly blackened (24–48 hours).

1. Salicin +.
   Nitrite −, indol −, gelatin +, motility +.
   Glucose +, lactose −, sucrose −.
   Spores ovoid, not usually abundant, excentric-subterminal, swelling rod. (Iron milk softly coagulated (2–5 days), first blackened (5–7 days), clot slowly digested (10–20 days).)
   Differentiated by antitoxin.
   Clostridium botulinum A and *B*

2. Salicin −.
   a. Indol −.
      Nitrite −, gelatin +, motility +.
      Glucose +, lactose −, sucrose −.
      Spores ovoid, abundant, excentric-subterminal, swelling rod.
      (Iron milk not coagulated, translucent then flocculent precipitate, rapidly blackened (24–48 hours), rapidly digested (8–10 days).)
      (1) Tyrosine crystals not observed.
      Clostridium sporogenes
      (2) Tyrosine crystals in old cultures.
      Clostridium tyrosinogenes
   b. Indol +.
      Nitrite −, gelatin +, motility +.
      Glucose +, lactose −, sucrose −, salicin ±.
      Spores ovoid, abundant, central-excentric, not markedly if at all swelling rod.
      (1) Pathogenic.
      Clostridium sordellii
      (2) Non-pathogenic.
      Clostridium bifermentens
      (*Clostridium centrosporogenes*)

B. Lead acetate: no blackening, no browning.
   Nitrite −, indol −, gelatin +, motility +.
   (Wine-red color in iron gelatin (24–48 hours).)
   Glucose −, lactose −, sucrose −, salicin −.
   Microaerophilic.
   Spores ovoid, abundant, excentric-subterminal, swelling rod.
   Clostridium histolyticum

V. Iron milk: no gaseous fermentation, no digestion, no blackening, coagulation late if any (15–20 days or more).
   Lead acetate: not blackened, but showing smoky browning at 24–48 hours, not measurably increased on incubation.
   Nitrite −, indol ±, gelatin +, motility +.
   Glucose −, lactose −, sucrose −, salicin −.
   Spores spherical, not abundant, terminal, swelling rod.
   A. Toxic.
   Clostridium tetani
   B. Non-toxic.
   Clostridium putrificum

CHAPTER 29

THE GLANDERS BACILLUS

Family: *Parvobacteriaceae*. Tribe: *Pasteurelleae*. Genus: *Malleomyces*, Small, slender, gram-negative rods, sometimes developing into branched filaments. Non-motile, non-spore-forming. Growth on culture media slow. Carbohydrates attacked feebly if at all. Type species: *Malleomyces mallei.*

Glanders is a disease seen, as a rule, only in the solipeds (horse, mule, ass), but is occasionally transmitted to other domestic animals, to wild animals and to man. Early regarded by many as a spontaneous, non-infectious affection, the transmissibility of glanders was demonstrated in 1837 by Rayer,[1] who infected a horse by inoculating it with material from a case of glanders in a human subject. The causative bacillus was discovered in 1882 by Löffler and Schütz,[2] whose work was soon confirmed and extended by Kitt,[3] Weichselbaum[4] and others.

Morphology and Staining.—The glanders bacillus is a small rod, straight or slightly curved, usually with rounded ends, and often of irregular contour. Rather wide variations in size are observed; the average length may be taken as 2 to 5 μ and the average breadth 0.5 to 1 μ. It is often compared to the tubercle bacillus but is usually found to be somewhat broader. In culture the bacilli tend to be shorter and more uniform in size than those observed in pus smears. In pus they are sometimes found within the leucocytes but more often occur free. There is no special arrangement in such smears, but in culture the bacilli may occur in pairs and, in older cultures, produce filaments with swollen ends in which true branching may be observed. The bacilli are non-motile, non-encapsulated, and do not form spores.

Colonies on agar are small, round, convex and amorphous in consistency. They are translucent and yellowish in color and upon aging (eight to ten days) become more opaque and the center may become light brown. The growth on potato usually exhibits a characteristic appearance; clear, amber, honey-like colonies appear which may coalesce, and frequently the potato around the growth becomes tinged a greenish yellow, not unlike the discoloration produced by *Ps. pyocyanea*. On horse-blood agar the colonies are grayish green with browning of the medium but no hemolysis.

The glanders bacillus stains with the ordinary aqueous aniline dyes, though not very readily. Best results are obtained with stains containing alkali, or a mordant such as phenol (Löffler's alkaline methylene blue, Ziehl's carbol fuchsin). The bacilli are not acid-fast and are gram-negative.

[1] Rayer: Mem. Acad. Med., Paris, 1837, *6*:625.
[2] Löffler and Schütz: Deut. med. Wchnschr., 1883, *9*:197.
[3] Kitt: Münch. Tierärztneue-Sch. Jahresbericht, 1883–84.
[4] Weichselbaum: Wien. med. Wchnschr., 1885, *35*:665.

Cells from young cultures take the stain fairly uniformly, but those in older cultures stain irregularly, with a tendency to bipolar staining. Granules and coccus-like bodies within the cell take the stain somewhat more readily, and the bacilli not infrequently have a beaded appearance in stained preparations. Worley and Young[5] have shown that this irregular staining is due to the presence of lipid granules which do not stain with the usual dyes but may be demonstrated with Sudan black B or iodine-fuchsin.

Physiology.—Growth occurs on ordinary nutrient media but is poor and slow on primary isolation. Forty-eight hours' incubation is generally necessary for the appearance on semisolid media of colonies 0.5 to 1 mm. in diameter. Growth is materially enhanced by the presence of glycerol, but glucose is without effect. A slightly acid reaction is favorable and the optimum temperature is 37° C., though growth may occur over the range from 22° to 44° C. Growth on enriched media such as Löffler's serum medium or horse-blood agar is not markedly better than on glycerol agar.

Fig. 127.—*Bacillus mallei* in pure culture. The generally poor staining is apparent, and bipolar staining may be observed in some of the cells. Methylene blue; × 1250.

The glanders bacillus is quite inactive biochemically. With the exception of glucose, there is no action on the usual carbohydrates, and even the glucose fermentation is irregular and variable from strain to strain. Coagulated serum is not digested, but gelatin may be liquefied under appropriate circumstances by some strains at least. At temperatures at which gelatin remains solid, growth is sparse and no liquefaction is observed, but if cultures are incubated at 37° C. for twenty-six to forty days, the gelatin does not solidify on cooling. Indol is not produced, and nitrates are not reduced to nitrites; small amounts of hydrogen sulfide may be formed. Slight acid production sometimes occurs in milk, with coagulation by the tenth day of incubation and decolorization of the indicator in the lower part of the tube.

The bacillus is but slightly resistant to adverse physical and chemical agents, being readily killed by heat (55° C. for ten minutes) and bactericidal

[5] Worley and Young: Jour. Bact., 1945, *49*:97.

chemicals. Desiccation experiments have not given uniform results; it is said that cultures dried on threads remain viable for three or four weeks. Pure cultures appear to be more resistant to desiccation than the bacilli in the nasal secretions from diseased animals, for infected discharges are usually sterile within a few days. Cultures die out in four to six weeks but may be maintained by transfer on glycerol agar.

Classification.—The relation of the glanders bacillus to other bacteria is by no means clear. In certain respects, such as the occurrence of branching and the favorable effect of glycerol on growth, it resembles the *Mycobacteria* though it is not acid-fast. It has, in fact, been grouped under the *Actinomycetales* by some workers[6] in the genus *Actinobacillus*. In other respects it is related to the *Brucella* species with which it is grouped in the current Bergey classification. Generally termed *Bacillus mallei*, it was formerly designated *Pfeifferella* by Bergey (4th ed.) as a newly created genus. This generic name has now been discarded and a new one, *Malleomyces*, coined.

Pathogenicity for Lower Animals.—Under natural conditions the horse is chiefly affected, but cases are occasionally observed in the carnivora (cats, dogs, menagerie animals) and in goats and sheep. Swine and pigeons are slightly susceptible. Cattle and house rats are immune. Rabbits and guinea pigs are susceptible to experimental inoculation.

Glanders manifests itself in an acute and a chronic form, which run into one another, the latter frequently terminating in an acute attack. The acute form is ushered in usually by a chill and the appearance of a high temperature in advance of any local manifestation. In a few days the mucous membrane of the nose is inflamed and becomes studded with nodules, the lymphatic system becomes largely involved, and edematous swellings appear in various parts of the body. General symptoms become more grave, and death follows in from eight to thirty days (Nocard).[7] The mule, and especially the ass, suffer commonly from the acute disease. The chronic form is the more usual type in the horse (90 per cent of cases). A great variety of symptoms and lesions have been noted in the latter animal, and the disease pursues most diverse courses in different individuals. The nasal membrane is often affected, and there is a profuse and infectious catarrhal discharge. Cutaneous glanders is known by veterinarians as *farcy*, the thickenings of the superficial lymphatics being termed "farcy buds" or "farcy pipes." In all forms of glanders there is a tendency to the production of nodules, which soften and pass over into ulcers. The glanders nodule has been considered by some writers to be structurally similar to the nodule formed by the tubercle bacillus (p. 578) but most observers are agreed that the former is a degenerative rather than a proliferative formation, and that it is radically different from the tubercle.

Experimental inoculation with pure cultures has given positive results not only in the horse, in which the characteristic features of the disease are reproduced, but in guinea pigs, field mice and other small rodents. House mice and white mice show a high but not absolute resistance, in contrast

[6] Thompson: Jour. Bact., 1933, *26*:221.
[7] Nocard: *Les maladies microbiennes des animaux*. Paris. 2nd ed. 1898.

to the great susceptibility of field mice. The guinea pig responds to inoculation in a typical fashion, and has been utilized for differential diagnosis. Both in the natural and in the experimental infection the bacteria are found chiefly in the nasal secretions and in the contents of the young nodules; in the older ulcers they are relatively few in number. The blood, as a rule, contains glanders bacilli only in acute general infection.

Pathogenicity for Man.—Veterinarians and others having to do with the care of horses are the most liable to contract glanders. Freshly isolated cultures are highly virulent, and a number of fatal infections have occurred among laboratory workers. The acute form of the malady is the more common in man, most cases terminating fatally within two or three weeks, sometimes within a few days of their inception. As in the horse, the mucous membrane of the nostrils, although not invariably affected, is a place of predilection for the glanders nodules and ulcers. Occasionally the chronic form may appear and linger for months or even years, with spreading ulceration and other features closely resembling those observed in the horse. Recovery from chronic glanders may take place, or the disease may pass into the acute stage.[8]

Path of Entrance.—The avenue by which the glanders bacillus usually enters the body of the horse has not been clearly determined. The intact skin probably rarely, if ever, permits entrance, but a slight wound or injury offers a ready portal, as attested by experimentation. The mucous membrane of the nose, especially if slightly abraded, may become the portal of entry, as may the intact conjunctiva, which, as shown by Conte,[9] can be infected by contact with infectious material in two to four hours, sometimes in thirty minutes. Infection by inhalation must be rare, to judge from animal experiments, if, indeed, it ever occurs. According to Nocard, who made a special study of the mode of infection, penetration takes place by way of the alimentary tract in the great majority of cases. There is weighty experimental and other evidence in support of this view.

In man the alimentary tract is certainly not the ordinary channel of entrance; meat from glandered animals has been ingested without resulting infection. Inhalation likewise hardly enters into consideration. Probably infection through a scratch or other break in the skin is the usual origin of human cases.

Diagnosis.—In prebacteriological days chronic glanders in the horse was frequently separated from other diseases only with difficulty and a considerable measure of uncertainty. At present the diagnosis of glanders is greatly facilitated by: (1) guinea-pig inoculation; (2) the mallein test—(*a*) subcutaneous, (*b*) ophthalmic; (3) the agglutination method; (4) the complement-fixation test.

1. *Guinea-Pig Inoculation.*—A male guinea pig is injected intraperitoneally with fragments of diseased tissue, scrapings from ulcers, or some of the nasal discharge from a suspected animal. A positive reaction is shown by the testicles becoming red and swollen, usually on the second or third day—

[8] Glanders in man is reported only occasionally; for a recent report see Panja and Chatterjee: Indian Med. Gaz., 1943, *78:*150.

[9] Conte: Revue Vétérin., 1893, p. 568.

the Straus reaction.[10] Together with the orchitis (inflammation of the parenchyma of the testicle) there are severe general symptoms which usually culminate in twelve to fifteen days. Grayish nodules are often found in the spleen and other internal organs. The test is not absolutely specific, for Kutscher[11] and Nocard have shown that an analogous orchitis may be produced by other organisms besides the glanders bacillus. It is often, however, of value, especially when, for one reason or another, other tests are inapplicable.

2. *The Mallein Test.*—Mallein is the concentrated glycerol broth in which the glanders bacillus has grown; it is prepared in the same manner as tuberculin. The mallein reaction consists in a rise of temperature, accompanied by a pronounced local reaction, and in many cases, though not invariably, by more or less profound constitutional disturbances.

(*a*) Subcutaneous injection of mallein (the size of the dose varying according to the concentration) into a glandered horse is followed by the

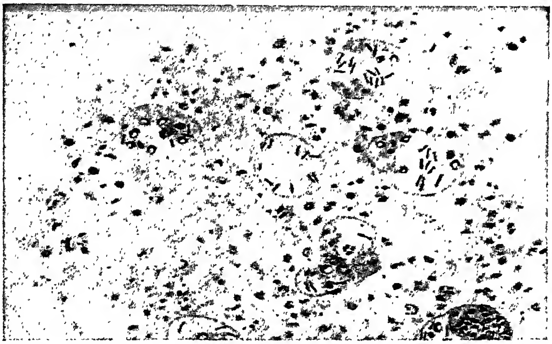


Fig. 128.—Glanders bacillus in giant cells and exudate of human lungs (Coleman and Ewing).

signs above noted, while in an animal not infected with glanders the temperature is slightly or not at all affected and the general symptoms are absent. The temperature of the suspected animal should be taken at two-hour intervals before the injection is made, and after the injection at the ninth, twelfth, fifteenth and eighteenth hours, at least. The increase of temperature in glandered horses varies from 1.5° to 2.5° C. above the normal, and is distinctly high on the second day after injection. Healthy horses often show a distinct temperature increase on the first day after inoculation, but as a rule this disappears quickly. In the use of mallein, as in the tuberculin test, care must be taken to exclude other influences that disturb the normal temperature relations. Experienced observers lay much stress upon the appearance of swelling at the seat of inoculation. In a glandered animal the tumefaction is large, hot and painful; it increases in

[10] Straus: Archives de Méd. Expér., 1889, *1*:489; Compt. Rend. Acad. Sci., 1889, *108*:530.

[11] Kutscher: Ztschr. f. Hyg., 1896, *21*:156.

size up to twenty-nine to thirty-six hours, persists for about a week, and gradually disappears. In a healthy animal a swelling may occur, but it is never large and vanishes within twenty-four hours.

There is complete agreement among veterinarians regarding the high diagnostic value of mallein. The reaction is specific, is usually sharp and decisive in character, and almost never fails to reveal the presence of infection. Nocard expressed himself very emphatically: "A complete mallein reaction is unequivocal; the animal that reacts is glandered. An animal which does not react to an injection of mallein is not glandered, whatever the character of the symptoms." Recent workers prefer the ophthalmic test to the subcutaneous test.

(*b*) The ophthalmic test consists in the introduction of the mallein, preferably in tablet form, into the conjunctival sac. The reaction is very reliable, and the test can be readily made by every practicing veterinarian.

3. *The agglutination method* (Macfadyen)[12] has had extensive official use in Prussia and Austria. The serum of normal horses agglutinates in dilutions of from 1:200 to 1:300, and the reaction is specific only when rather high dilutions (1:500 to 1:3200) are used. The serum from sound animals, however, sometimes agglutinates the glanders bacillus in a dilution as high as 1:500. Occasionally the reaction fails to appear in the serum of glandered animals. The test is liable to the usual difficulties and source of error in the hands of an unskilled observer. Moore[13] uses a suspension of *B. mallei* in carbolized salt solution prepared from a glycerol-agar culture, killed by heating to 60° C. for two hours.

4. *The complement-fixation test* is very accurate but demands special laboratory facilities and is less easy to apply in a practical way than the ophthalmic test.

Immunity and Prophylaxis.—Permanent immunity to glanders can neither be conferred by an attack of the disease nor produced by any artificial means. Nocard fed with infectious matter three horses which had previously recovered from the disease, and found that these animals showed no resistance superior to that of a healthy control animal. Similarly, Lobel, Schaaf and Roza[14] were unable to produce an immunity in guinea pigs or horses by the inoculation of bile-attenuated avirulent bacilli. Chronic glanders may exist for years, and is in no wise a warranty against the sudden development of an acute attack.

No very potent nor characteristic toxic substance has been obtained from cultures of the glanders bacillus, and attempts at immunization with the products of this organism have been eminently unsuccessful. It is stated by a number of observers that repeated injections of mallein will exercise a curative action upon certain forms of recent infection, but experimentally mallein is without immunizing power. The sera of animals treated with mallein injections and the sera of naturally immune animals, such as cattle, are, according to most observers, totally devoid of any preventive or curative value. The most that has been accomplished in the way of immuni-

[12] Macfadyen: Jour. Comp. Path. and Therap., 1896, *9*:322.
[13] Moore: Jour. Inf. Dis., 1907, Suppl. No. 3, p. 85.
[14] Lobel, Schaaf and Roza: Nederland. Indische Blad. Diergeneesk., 1941, *53*:100.

zation is a very moderate augmentation of resistance in dogs injected with small non-fatal doses of living cultures.

The experience of Great Britain shows that the disease may be practically eradicated by slaughtering every animal showing clinical signs of glanders or giving a positive mallein test, and properly disposing of the carcass. By this means the number of horses affected was cut down from 2012 in 1906 to 2 in 1925.

BACILLUS WHITMORI (BACILLUS PSEUDOMALLEI)

Melioidosis, a disease of rodents somewhat similar to glanders, is caused by *Bacillus whitmori*, a microorganism which closely resembles the glanders bacillus. It has been observed in Rangoon, where it is thought to be primarily a disease of the wild rat and is occasionally communicable to man. *B. whitmori* differs from *B. mallei* in that it is actively motile, liquefies gelatin and attacks carbohydrates more energetically. It grows considerably more rapidly and its colonies on glycerol agar develop a wrinkled, corrugated surface and are quite different in appearance from those of *B. mallei.* A second colony type may be produced, however, which is very similar to the colonies of the glanders bacillus. *B. whitmori* is thought by some to be closely related to *Pseudomonas pyocyanea* in many respects and strains have been reported[15] which produce pyocyanin.

The characteristic lesion of the disease is a small caseous nodule which is found in man in almost any part of the body except the brain. The nodules may coalesce to form large areas of caseation in some cases and in others break down into abscesses. There is some reason to believe that melioidosis in man is often traumatic in origin, either the bacilli entering the injured tissues or the defense mechanism being broken down in part by the injury.[16] Rodents usually die within a short time from septicemia, and the lesions appear as small nodules superficially resembling tubercles. Guinea pigs and rabbits are highly susceptible to inoculation. *B. whitmori* also produces the Straus reaction.

[15] Blanc, Delage and Martin: Ann. Inst. Pasteur., 1943, *69:*65.

[16] *Cf.* Le Moine, Hasle and Nguyen-Duc-Khoi: Bull. Soc. Med.-Chirurg. Indochine, 1937, *15:*662; Toullec and Huard: *ibid.*, 1937, *15:*667; Sudibyo: Geneesk. Tijdschr. v. Nederl.-Indie., 1938, *78:*1424 (English summary).

CHAPTER 30

CORYNEBACTERIUM (THE DIPHTHERIA BACILLUS)[1]

Order: *Actinomycetales*. Family: *Mycobacteriaceae*. Genus: *Corynebacterium*. Gram-positive rod-like forms, slender and often slightly curved. Club-shaped forms frequently observed, and branching forms present in old cultures. Staining irregular, often in segments to give a barred appearance. Non-motile. Aerobic. Non-spore-forming. Powerful exotoxin produced by some species. Characteristic snapping motion when cells divide. Type species: *Corynebacterium diphtheriae*.

As a clinical entity diphtheria dates from the observations of Bretonneau in 1826. The diphtheria bacillus was observed and described by Klebs[2] in 1883, but its etiological relation to the disease was suggested by the investigations of Löffler[3] the following year. Löffler isolated the bacillus observed by Klebs in pure culture from a number of cases of diphtheria but expressly disclaimed the assumption that his bacillus was the causal agent of diphtheria, in part because he found it in the throat of a healthy child, and in part because he did not find it in all cases of what were apparently clinical diphtheria. The significance of Löffler's findings is now clear, however, for it is known that other bacteria, such as streptococci, can produce a condition in the throat closely resembling diphtheria and that the diphtheria bacillus is not infrequently present in the throats of healthy carriers. Further investigations by other workers indicated that the Klebs-Löffler bacillus was always present in the typical false membrane of diphtheria. In 1888 Roux and Yersin[4] showed that this bacillus formed a soluble toxin which reproduced the characteristic symptoms and lesions of diphtheria and thus demonstrated its etiological relation to the disease.

Morphology and Staining.—The diphtheria bacillus is a slender rod ranging from 1 to 6 μ in length and 0.3 to 0.8 μ in breadth. The bacilli are highly pleomorphic, for, in addition to the straight or slightly curved rods, club-shaped and branching forms are not infrequently observed. The presence of the latter, which are a consequence of true branching, is indicative of the close relation of the diphtheria bacillus to some of the higher fungi, and it is classified with the actinomycetes rather than under the *Eubacteriales* or "true bacteria." Upon completion of cell division a movement designated as snapping occurs, and the bacilli may remain attached but at sharp angles to one another.

[1] Diphtheria is considered in detail by Andrewes *et al.*: *Diphtheria: Its Bacteriology, Pathology and Immunology*, Medical Research Council (British), London, 1923; and by Forbes: *Diphtheria, Past and Present; Its Aetiology, Distribution, Transmission and Prevention*, John Bale, Sons & Danielsson. London. 1932. See also Corson: *Diphtheria: A Summary of Recent Literature*, Bull. Hyg., 1943, *12*:969.

[2] Klebs: Verhandlungen des Congresses f. innere Med., 1883.

[3] Löffler: Mitt. a. d. k. Gesund., 1884, *2*:421.

[4] Roux and Yersin: Ann. Inst. Pasteur, 1888, *2*:629.

The diphtheria bacillus exhibits a marked tendency to stain irregularly. Some cells stain solidly, others take the stain more deeply in transverse bands to give a barred appearance, and in still others deeply staining metachromatic or Babes-Ernst granules are found. A single cell may contain from one to generally not more than five or six such metachromatic granules; they may be found at one or both ends of the cells, particularly those with swollen ends, and when more than two are present the remainder are scattered within the cell substance. This irregular staining is apparent with Löffler's alkaline methylene blue or with toluidine blue; Neisser's stain[5] is regarded by many workers as producing an even greater contrast between the heavily and lightly staining portions of the cell substance. Morton and Francisco[6] have shown that the metachromatic granules are more readily differentiated when the basic dye is made up in an acid solution.

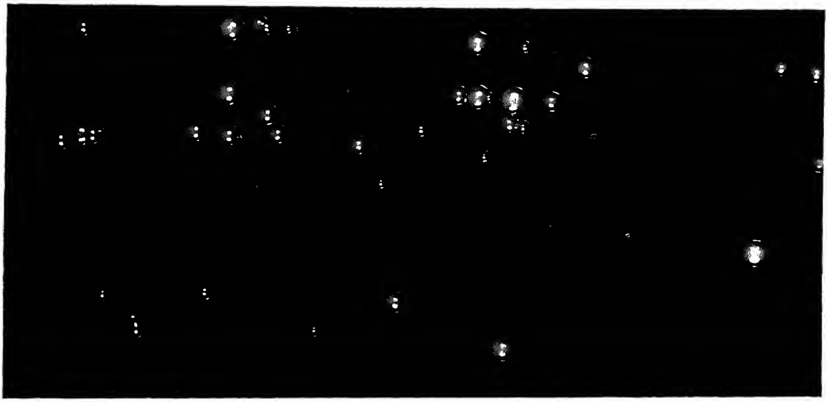


Fig. 129.—Colonies of *Corynebacterium diphtheriae* on blood agar. Note the smooth, raised translucent appearance and relatively small size. × 2.

In stained smears the appearance of diphtheria bacilli is highly characteristic. They may not be identified on morphological grounds alone, however, for many of the pseudodiphtheria bacilli or diphtheroids stain in the same irregular fashion and are similarly pleomorphic. It was formerly thought that there was an association between morphological type and virulence, and Wesbrook[7] divided the diphtheria bacilli into a series of morphological types, generally known as the Wesbrook types. At present, however, little emphasis is placed upon morphology in this connection, though in a general way granular types seem to predominate in clinical diphtheria and there appears to be a rough association between morpho-

[5] The bacilli are stained with an acid methylene blue solution followed by Bismarck brown.

[6] Morton and Francisco: Stain Technol, 1942, *17*:27.

[7] Wesbrook, Wilson and McDanile; Trans. Assn. Amer. Physicians, 1900; see also Gorham: Jour. Med. Res., 1901, *6*:201.

logical type and the *mitis* and *gravis* types, as will appear. The diphtheria bacillus is gram-positive but decolorizes more readily than most of the gram-positive bacteria.

Surface colonies on Löffler's serum medium or on agar are small and gray; when viewed under low magnification they are found to be coarsely granular and somewhat irregular in outline, with ragged or fringed edges. On differential media containing potassium tellurite, colonies of the diphtheria bacillus are dark gray or black because of reduction of the tellurite and readily differentiated from those of contaminating bacteria. Tellurite reduction apparently occurs within the bacterial cell.[8] It may be noted that although the characteristic morphology of the diphtheria bacillus is apparent in stained smears from colonies on Löffler's medium, smears from colonies on tellurite media are often not characteristic.

Physiology.—The optimum growth temperature for the diphtheria bacillus is 34° to 36° C. and it grows well at 37° C.; growth will take place

Fig. 130.—The diphtheria bacillus, *gravis* strain, pure culture on blood agar. Methylene blue stain. Note the bipolar staining and the club-shaped forms. The lightly stained cells with deeply stained areas are characteristic of *gravis* morphology. × 1200.

over the range from 15° to 40° C. An alkaline reaction is required, *p*H 7.8 to 8.0, and free access to air is essential, for growth under anaerobic conditions is sparse.[9]

In primary isolation the diphtheria bacillus is best cultivated on enriched media. Growth is rapid on Löffler's serum medium (3 parts of beef or sheep serum and 1 part of 1 per cent dextrose broth coagulated in slant form by inspissation) and minute but visible colonies appear after twelve to twenty-four hours' incubation. In recent years a variety of differential and selective media have been introduced, all of which contain potassium tellurite. The better known of these are the chocolate agar-tellurite medium

[8] Morton and Anderson: Proc. Soc. Exp. Biol. Med., 1941, *46*:272.

[9] Strains of virulent diphtheria bacilli which grow more luxuriantly under anaerobic conditions than in the presence of air have, however, been reported. *Cf.* Emilio: Giorn di Batteriol. e Immunol., 1938, *21*:256.

of Anderson and his co-workers,[10] which has been subject to minor modifications by a number of other workers, such as Neill's medium and Hoyle's medium which are used in England,[11] and the various media developed by Clauberg,[12] whose inspissated serum-glycerol-tellurite medium has been widely tested. There is general agreement that the proportion of positive cultures is somewhat higher with the Clauberg medium than with Löffler's medium; whether the heated blood-tellurite media are superior to Löffler's medium is not clear. As indicated above, the characteristic morphology of the diphtheria bacillus is not always seen in smears from colonies on tellurite media and, therefore, in some laboratories both Löffler's medium and a differential tellurite medium are inoculated and the former used for microscopic examination if typical colonies appear on the differential medium.

Enriched media are not, however, essential to the growth of the diphtheria bacillus, for this microorganism can be cultivated on ordinary nutrient and infusion media. Growth is somewhat scanty on the former but good on the fresh meat infusions. The intensive investigations of Mueller[13] have shed considerable light on the growth requirements of these bacilli. Some strains, including the well-known Park 8, may be cultivated on synthetic solutions containing a number of amino acids together with small quantities of nicotinic acid, β-alanine or pantothenic acid, and pimelic acid. It has been suggested[14] that pimelic acid is utilized by this bacterium for the synthesis of biotin since growth is stimulated by biotin in the absence of pimelic acid. Recently isolated strains also require oleic acid for development, especially if the inoculum is small.[15] Nutritive requirements differ somewhat from one strain to another and a general statement is not possible.

The diphtheria bacillus does not liquefy gelatin or digest coagulated protein. Indol is not formed[16] and nitrates are reduced to nitrites. All strains form acid but no gas from dextrose and levulose, and some strains ferment dextrin, glycogen, starch, galactose, maltose and glycerol. There appear to be no well-defined biochemical groups among these bacilli.[17] The fermentation of dextrose is of some interest in that propionic acid is formed.[18] Other products of the fermentation include lactic, acetic, formic and succinic acids and ethyl alcohol.

In ordinary culture media the diphtheria bacillus may retain its vitality for relatively long periods of time. It will live six to eight weeks on agar, five to six months on blood serum, twelve to fifteen months on dextrose

[10] Anderson, Happold, McLeod and Thomson: Jour. Path. Bact., 1931, *34*:667.

[11] For example, see Hynes: Jour. Path. Bact., 1942, *54*:138.

[12] Clauberg: Centralbl. f. Bakt., 1929, *114*:539; *ibid.*, 1931, *120*:324; *ibid.*, 1933, *128*:153; *ibid.*, 1935, *134*:271; *ibid.*, 1936, *135*:529.

[13] Summarized by Mueller: Jour. Bact., 1938, *36*:499; Bact. Rev., 1940, *4*:97.

[14] duVigneaud, Dittmer, Hague and Long: Science, 1942, *96*:186.

[15] Cohen and Mueller: Jour. Bact., 1941, *41*:58.

[16] The test with sulfuric acid and potassium nitrite may be positive because of the formation of indol-acetic acid, but no color is produced with Ehrlich's reagent, *p*-dimethylamidobenzaldehyde. (Frieber: Centralbl. f. Bakt., 1921, *87*:254.)

[17] Frobisher: Amer. Jour. Hyg., 1938, *28*:1.

[18] Tasman and Brandwijk: Jour. Inf. Dis., 1938, *63*:10; *ibid.*, 1940, *67*:282.

blood serum, and as long as three months in particles of diphtheritic membrane. Although virulence is ordinarily reduced by continued culture on laboratory media, some strains remain fully virulent, *i.e.*, toxigenic, on prolonged cultivation; Löffler recorded one instance in which virulence was maintained over 77 transfers covering a period of twenty-seven months. The bacilli are unusually susceptible to heat; a suspension or broth culture is killed by holding at 58° C. for ten minutes. In diphtheritic membrane they are considerably more resistant.

Toxin.—With the possible exception of the Shiga dysentery bacillus, the diphtheria bacillus is the only aerobic bacterium that produces a powerful exotoxin comparable to those formed by the sporulating anaerobes. Filtrates from broth cultures are not so toxic as those of the tetanus and botulinus bacilli; exceptionally potent filtrates may contain as much as 1000 guinea-pig MLD's per cubic centimeter. It may be noted that in

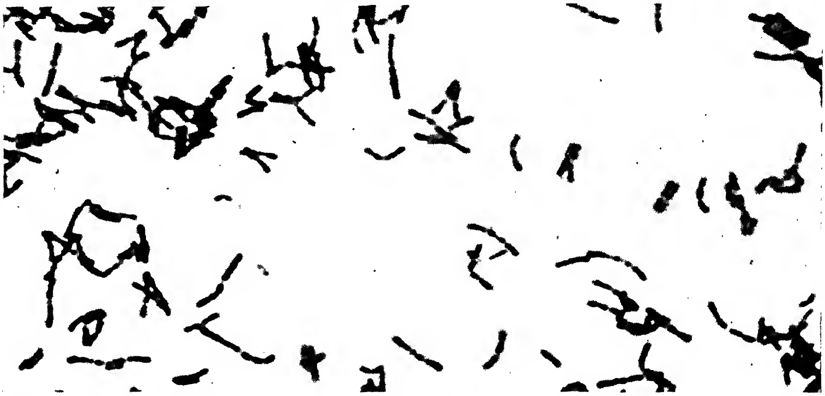


Fig. 131.—The diphtheria bacillus, *intermedius* strain, pure culture on blood agar. Methylene blue stain. Note the irregular staining and barred appearance characteristic of the *intermedius* variety. × 1200.

regard to the diphtheria bacillus virulence and toxigenicity are synonymous. *The virulence test*, the inoculation of guinea pigs with broth culture of the bacillus, is, then, a test of the ability of the bacilli to form toxin.

The production of toxin by the diphtheria bacillus is markedly influenced by environmental and nutritive conditions; even strongly toxigenic strains may produce little or no toxin under unfavorable conditions. A slightly alkaline reaction, *p*H 7.8 to 8.0, is essential, for an acid reaction strongly inhibits toxin formation. Free access to air is also necessary, and for the production of toxin the bacilli are cultivated in thin layers of beef infusion broth. Maximum amounts of toxin are found after seven to ten days' incubation at 36° to 37° C.

An infusion medium, beef infusion, containing adequate amounts of peptone (2 per cent) has long been regarded as essential to the production of maximum amounts of toxin. The marketed brands of peptone are

variable in this respect, some being much better for the production of toxin than others. The presence of protein or large peptone molecules is not essential to toxin formation, however, and potent toxins may be produced in chemically defined media containing appropriate amino acids and other compounds. The critical factor is not the quality of peptone or other source of nitrogen, as once thought, but the concentration of iron in the medium. Pappenheimer and Johnson[19] found that maximum toxin production occurs only over a narrow range of iron concentration, the optimum being 0.14 gm./ml., and 5.0 gm./ml. almost completely inhibits its formation. Calcium, sodium and potassium also affect toxin production but the concentrations are not so critical. Mueller[20] has suggested that the small amount of toxin produced in the presence of large amounts of iron represents normal production while the increased production under conditions of iron starvation is possibly the result of a compensatory mechanism in which the toxin molecule takes part in some process ordinarily catalyzed by an iron-containing enzyme. However this may be, potent diphtheria toxin is formed in a reproducible semi-synthetic medium containing hydrolyzed casein, nicotinic and pimelic acids, cystine, maltose, calcium and iron that gives better and more consistent toxin production than the complex infusion media.[21]

The properties of the diphtheria toxin are similar to those of the other soluble toxins which have been discussed elsewhere (p. 173) and need not be considered at length here. Suffice it to say that the toxin is unstable to slight acidities, *i.e.*, a *p*H of 6 or less, is heat-labile and apparently protein in nature. The production of toxin in synthetic nutrient solutions containing insignificant amounts of high molecular weight substances allows its separation in a relatively pure form by salting out and dialysis. A protein in a high state of purity and having a molecular weight of about 72,000 has been prepared from such cultures by Pappenheimer[22] and is regarded by him as identical with diphtheria toxin. The guinea-pig MLD of this material is about 0.001 mg.

Diphtheria toxin is an excellent antigen and gives rise to high titer antitoxic sera. The standardization of diphtheria toxin and antitoxin is discussed elsewhere (p. 256). It has been noted from time to time that various substances, including bile, ascorbic acid (vitamin C), sterols such as lanolin, cholesterol and the like, will neutralize diphtheria toxin. The significance of such observations is not entirely clear. Generally relatively large amounts are required to neutralize small quantities of toxin. One or two attempts have been made to utilize ascorbic acid in the treatment of clinical diphtheria but without significant results.

It may be noted that there is great variation from strain to strain of diphtheria bacilli in the ability to produce toxin, ranging from the highly toxigenic strains to relatively or completely atoxigenic ones. The well-

[19] Pappenheimer and Johnson: Brit. Jour. Exp. Path., 1936, *17*:335.
[20] Mueller: Jour. Immunol., 1941, *42*:343.
[21] Mueller and Miller: Jour. Immunol., 1941, *40*:21.
[22] Pappenheimer: Jour. Biol. Chem., 1937, *120*:543; see also Linggood: Brit. Jour. Exp. Path., 1941, *22*:255.

known Park No. 8 strain is one of the good toxin producers and has been widely used for this purpose. It has been observed by some workers that pure cultures of toxigenic strains will, at times, give rise to weaker toxin producers or completely atoxigenic varieties.[23]

Variation.—As in other groups of bacteria, smooth and rough variants of the diphtheria bacillus have been observed and the type of colony formation has been found to be correlated with morphology and virulence; the S variant is the more virulent and the form commonly found in acute cases of diphtheria. Morphological and biochemical variation observed in the diphtheria bacilli has been critically reviewed by Morton.[24] As a consequence of its pleomorphism and tendency to branching and the like, the diphtheria bacillus has been thought by some to undergo the cyclic transformations of a complex life cycle including the production of gonidial and filterable forms. This concept is not generally accepted. It has also been suggested that some of the diphtheroid bacilli are diphtheria bacilli which have lost their ability to form toxin.

Types.[25]—Particularly interesting types of the diphtheria bacillus, perhaps related to the S and R variants, were described by Anderson and others[10] in Leeds, England, and have since been observed by a number of other workers in various parts of the world. The varieties designated as the *gravis* and *intermedius* types have been found usually, though not always, associated with severe cases of diphtheria, while the *mitis* type is said to be isolated in larger proportion from the milder cases. This association with the clinical type of diphtheria appears to be definite and marked in England but less so on the Continent. In this country there appears to be little or no such association and the *mitis* type is not infrequently found to be the cause of severe or malignant diphtheria. The *mitis* type occurs much more often in the United States; Frobisher[26] estimates that only 1 per cent of the strains are the virulent *gravis* type.

These types may be differentiated by their colonial form on tellurite media. The *gravis* type produces irregular striated colonies predominantly gray in color; the *mitis* type, small, round, smooth, convex colonies predominantly black in color and softer in consistency; and the colonial form of the *intermedius* type lies between these. Colonial differences are also apparent on certain other media, such as trypsin-serum agar[27] and a potato extract-cystine-water blue-glycerol medium devised by Clauberg.[28] On fresh blood agar the *mitis* type is usually hemolytic, the *intermedius* type non-hemolytic, and the *gravis* type usually non-hemolytic. A further distinction is the fermentation of glycogen and starch by the *gravis* type; but other biochemical tests do not differentiate these types.

There is some association between colonial type and the morphology of the bacillary forms. Those of the *gravis* type show one or two deeply stain-

[23] Cromwell: Jour. Bact., 1926, *11*:65.

[24] Morton: Bact. Rev., 1940. *4*:177.

[25] See the comprehensive review by McLeod: Bact. Rev., 1943, *7*:1.

[26] Frobisher: Amer. Jour. Pub. Health., 1942, *32*:709.

[27] Dudley, May and O'Flynn: Medical Research Council (British) Spec. Rept. Series No. 195, 1934.

[28] Clauberg: Centralbl. f. Bakt., 1938, *142*:478.

ing areas, the remainder of the cell staining very lightly; metachromatic granules are seldom observed. Bacilli of the *mitis* variety stain irregularly and contain very many well-developed metachromatic granules. The *intermedius* forms exhibit the familiar barred appearance. While some 80 per cent of the *intermedius* variety conform to this morphology, only 50 to 60 per cent of the *gravis* strains are typical, the remainder resembling the *mitis* and *intermedius* forms. Furthermore, 5 to 20 per cent of the *mitis* strains show barred forms.

Serological investigation has shown that while these three types are antigenically distinct from one another, the types are not necessarily

Fig. 132.—The varieties of the diphtheria bacillus on chocolate-tellurite agar. Left, *mitis* type; note the characteristic raised, small black colony. Center, *intermedius* type; the lighter color, beginning radial striation and small size are apparent. Right, *gravis* type; the gray color, larger size, raised center and radial striation are evident.

homogeneous. The *mitis* strains are heterogeneous, the *gravis* strains fall for the most part into two types, and the *intermedius* strains are relatively homogeneous, some strains showing relationship to the *gravis* types.

Not all strains showing the morphological and biochemical characteristics of these types are virulent, *i.e.*, toxigenic, diphtheria bacilli. Since the diphtheria bacillus is differentiated on the basis of the formation of immunologically specific toxin, it is apparent that *mitis*, *gravis* and *intermedius* types of diphtheroid bacilli occur. In the series studied by Frobisher,[26] for example, only 10 per cent of the strains typed as *gravis* were toxigenic. Furthermore, a certain proportion of toxigenic strains cannot be allocated into one or another of these types. The proportion of indeterminate strains is said to be higher when diphtheria is mild. Anderson *et al.*[10] found about 5 per cent of their strains (British) were not typable and 44 per cent of the strains found by Seligmann[29] in New York City in 1940 were indeterminate.

[29] Seligmann: Amer. Jour. Hyg., Sec. B, 1941, *34*:125.

The apparent association of the *gravis* and *intermedius* types with the more severe infections has not as yet been explained. The toxins produced by the three varieties are equally neutralizable by the ordinary antitoxic sera, but the *mitis* strains appear to be the more highly toxigenic and produce toxin more actively *in vitro* than the *gravis* or *intermedius* forms. While the three types appear to be equally virulent for guinea pigs, infections with *gravis* or *intermedius* strains have been reported[30] to be more often fatal to rabbits than *mitis* infections. Mueller[31] has observed that the concentration of iron in the throat is too high for maximum toxin production but that a *gravis* strain produced more toxin in the presence of the excess than did three non-*gravis* strains.

Although this differentiation of the diphtheria bacilli appears highly suggestive, it is difficult to assess the significance of the apparent asso-

CHARACTERISTICS OF THE DIPHTHERIA BACILLUS TYPES

Type			*mitis*	*intermedius*	*gravis*
Morphology	Microscopic		Usually long, with many metachromatic granules—80 per cent typical	Usually barred, club forms common—80 per cent typical	Short, evenly staining —50–60 per cent typical
	Colonial	Tellurite	Small, round, smooth, convex, black with greyish periphery	Small, flat, dull, grey, raised center	Large, irregular, dull, grey, raised center, radial striations
		Chocolate	Smooth, semi-opaque, glistening	Flat, dry, opaque, slight greenish zone	Flat, dry, matt, opaque
		Broth	Uniform turbidity, sometimes slightly granular, soft pellicle	Finely granular turbidity	Granular, flakes, pellicle—variable
Physiology	Fermentation of	Glycogen	−	−	+
		Starch	−	−	+
	Hemolysis		+	−	±
Immunology			Heterogeneous	Relatively homogeneous	Two main types

ciation of certain types with varying degrees of severity in the clinical manifestations of the disease.

Pathogenicity for Man.—Diphtheria in man is usually a local infection of the mucous surfaces. The pharynx is most commonly affected, but infection of the larynx, or membranous croup, and nasal diphtheria, or membranous rhinitis, are not infrequently observed. Diphtheritic infections of the conjunctiva and of the middle ear are less common and cutaneous or wound diphtheria is only occasionally observed. Infection of the mucous surfaces of the genital organs is occasionally found. The invasion of other localities is rare; primary infection of the lungs and diphtheritic meningitis have been observed, and infection of the umbilicus in the newborn has been reported.

30 Murray: Jour. Path. Bact., 1935, *41*:97.
31 Mueller: Jour. Immunol., 1941, *42*:353.

It has long been known that diphtheria bacilli are not often found in the internal organs, but the frequency with which such infection occurs is not definitely known. Diphtheria bacillus septicemia is occasionally observed,[32] however, and it is of interest to note here that a few cases of acute vegetative endocarditis caused by the diphtheria bacillus have been reported.[33]

The symptoms and lesions produced are due partly to the presence of the bacillus and partly to its toxin. The chief local consequence of infection is a degeneration of the epithelial cells, extending to the underlying tissues and accompanied by a profuse fibrinous exudation, and the characteristic diphtheritic membrane, containing fibrin, dead tissue cells, leucocytes and bacteria, is formed on the affected surface. The mechanical interference of the membrane with breathing may assume significant proportions and even necessitate intubation or tracheotomy.

Although diphtheria toxin undoubtedly plays a part in the formation of the membrane, its systemic effects following absorption are by far the most important, and diphtheria is, like tetanus, essentially a toxemia. The organs most severely affected are the kidneys, heart and nerves. A variety of lesions may be found in the kidneys, an acute interstitial nephritis being the most common. The lesions in the heart consist commonly of a fatty degeneration in the muscle fibers, which may be very extensive. Fatty degeneration also occurs both in the myelin sheath of the peripheral nerves and in the white matter of the brain and cord. These changes in muscle and nerve account for the serious cardiac weakness often observed in diphtheria and the frequent occurrence of the more or less extensive paralysis which so commonly follows an attack of the disease. It is probable that a small amount of toxin can cause extensive damage in these tissues.

Pathogenicity for Lower Animals.—Diphtheria is not a natural disease of lower animals. There is a popular belief that cats may become infected and disseminate the bacilli, but this is not true. Both the local and general symptoms of human diphtheria in man can, however, be reproduced by animal inoculation. Inoculations upon the healthy mucous membrane of most adult animals lead to no changes, but if young animals be injected intratracheally, or if the mucous surface be injured before inoculation, a characteristic false membrane is produced which is histologically identical with that found in man.

The subcutaneous inoculation of a guinea pig with a sufficient amount of a young broth culture or toxic filtrate will produce death in one to four days, the time depending upon the size of the inoculum. The animal becomes obviously ill twelve to eighteen hours after inoculation, and nephritic symptoms, paralytic manifestations and other characteristics of human diphtheria are often observed. Postmortem findings include an edema and possibly necrosis at the site of inoculation, congestion of the regional lymphatics and abdominal viscera, a pleural exudate and, characteristic of diphtheritic toxemia in this animal, an enlarged and hemorrhagic condition of the adrenals. As a rule, the bacilli remain localized and

[32] The literature is reviewed by Kaschel: Ztschr. f. Kinderheilk., 1938, *59*:437.
[33] *Cf.* Buddingh and Anderson: Arch. Int. Med., 1937, *59*:597.

are not found in large numbers in the internal organs of the infected animal. Guinea pigs receiving smaller doses that do not die by the fourth day may develop paralytic symptoms and cachexia and die later on, a condition obviously different from the acute toxemia.

Animals vary considerably in their susceptibility to infection. Rats and mice are relatively refractory; rabbits are less susceptible than guinea pigs; cats, dogs and pigeons are highly susceptible. Paralytic manifestations appear more frequently in dogs and pigeons than in guinea pigs or rabbits. Frobisher[34] has found that young chicks are susceptible to virulent diphtheria bacilli and their toxin and may be used instead of guinea pigs for the virulence test.

Bacteriological Diagnosis of Diphtheria.—To establish a diagnosis of infection with diphtheria bacilli, in either case or carrier, the bacillus must be isolated and its toxigenicity demonstrated. The specimen is taken on a swab, either plain or previously dipped in sterile horse serum which is coagulated on the surface by twirling in a flame. It is best to inoculate two media, Löffler's serum agar and a tellurite medium such as chocolate tellurite agar; if only a single medium can be used, tellurite is preferable. A blood agar plate should be inoculated as well, both for the isolation of diphtheria-like colonies and to provide for the cultivation of hemolytic streptococci which may be present. After the plates have been inoculated a smear may be made by rolling the swab on a slide, and stained with alkaline methylene blue; it will serve to show the presence of the spirochetes and fusiform bacilli of Vincent's angina should these be present.

Diphtheria bacilli grow up in eighteen to twenty-four hours' incubation. If the characteristic black or grey colonies appear on tellurite, smears may be made from such colonies and from the Löffler slant for microscopic examination; the morphology of the diphtheria bacillus is frequently not characteristic on tellurite as indicated above. It is often inferred that only diphtheria bacilli grow as black colonies on tellurite medium. This is not true for any bacterium that reduces tellurite will produce similar colonies; tellurite-reducing bacteria, other than diphtheria bacilli, from the nose and throat are usually staphylococci or micrococci and as a rule their colonies resemble those of the *mitis* variety of diphtheria bacillus but are blacker.

If morphologically typical bacilli are found toxigenicity must be tested by animal inoculation. This is ordinarily carried out in the guinea pig by subcutaneous or intracutaneous inoculation. In the first instance the growth from a Löffler slant is suspended in 10 ml. saline and 4 ml. injected subcutaneously into each of two guinea pigs, one of which has received 250 units of diphtheria antitoxin twenty-four hours previously. The diphtheria bacillus will kill the unprotected pig in three to five days and autopsy will show local edema and the characteristic hemorrhagic enlarged adrenals, while the protected animal will survive. For the intracutaneous test the growth from a Löffler slant is suspended in 20 ml. saline and 0.15 ml. injected into the shaven abdominal skin of each of two pigs as above. Toxigenicity is indicated by the development of a local infiltrated lesion

[34] *Cf.* Frobisher, Parsons and T'ung: Amer. Jour. Hyg., 1942, *35*:381.

which shows superficial necrosis in two or three days in the unprotected pig. By the latter technique a number of tests may be carried out in the same pair of animals.

Immunity.—Immunity to diphtheria, arising as a consequence either of recovery from a frank attack of the disease or of inapparent infection, is essentially an antitoxic immunity. Antibacterial substances appear to be of little significance and the refractory state is associated with the presence of antitoxin in the blood serum and body fluids.

The Schick Test.—Immunity to diphtheria, then, may be measured by the amount of circulating antitoxin present in a given individual. A skin test has been devised by Schick,[35] and is known as the *Schick test,* in which a minute amount of diphtheria toxin is injected intradermally. In the nonimmune the irritant action of the toxin gives rise to a local erythema followed by necrosis and desquamation, and the reaction is said to be positive. (See the Römer reaction, p. 257.) In the immune, however, the toxin is neutralized by the antitoxin that is present, the characteristic reaction does not develop, and the reaction is negative. The amount of toxin injected is usually 1/50 of a guinea-pig MLD in a volume of 0.1 or 0.2 cc.; the Permanent Standards Committee of the League of Nations specifies 1/40 MLD in 0.2 cc. and 1/50 MLD in 0.1 cc.[36] According to Moloney and Taylor,[37] however, a toxin of three times this strength gives more sharply defined reactions. Considerable interest has attached to diluents for the Schick toxin since it is not stable in phenol-saline solutions. The dilute toxin is, however, stable in 2 per cent peptone solution, a borate buffer-gelatin solution, and a glycerol-gelatin solution which has been proposed recently. The advantages of a ready-diluted Schick toxin are obvious, and toxins diluted ready for use are now generally available.

For many years a negative Schick test has been regarded as indicating the presence of 1/20 unit or more of antitoxin per cubic centimeter in the blood serum and a positive test less than 1/40 unit. More recent experiments,[38] however, have indicated that the so-called "Schick level" of immunity is much lower than this and in the neighborhood of 1/250 to 1/500 unit of antitoxin; negative reactions have been obtained in persons with as little as 0.0005 unit. Phair[39] has expressed the opinion held by a number of workers that a negative Schick test is indicative not only of antitoxin content of the blood but also involves a defense mechanism other than that of antitoxin production.

A scarification test in which diphtheria toxin is introduced by punctate scarification rather than intradermal injection has been introduced by Reh and is called *Reh's test.* It is said to be somewhat simpler to perform

[35] Schick: Münch. med. Wchnschr., 1913, *60*:2608.

[36] Report of the Permanent Commission on Biological Standardization, League of Nations Health Organization. London. 1931.

[37] Moloney and Taylor: Jour. Immunol., 1937, *33*:191. See also Cameron and Gibbard: Canadian Jour. Pub. Health, 1941, *32*:83.

[38] *Cf.* Glenny and Waddington: Jour. Path. Bact., 1929, *32*:275; Fraser and Halpern: Trans. Roy. Soc. Canada, Sec. V, 1935, *29*:53; Parish and Wright: Lancet, 1935; *i*:600; *ibid.*, 1938, *i*:882.

[39] Phair: Amer. Jour. Hyg., 1942, *36*:283.

than the Schick test and, when carried out with a potent toxin (with a guinea-pig MLD of 2000 per cc.), to give parallel results with the Schick test.[40] Recently introduced (1934), it has not been generally used.

The question of whether the Schick test is indicative of a degree of immunity such that subsequent infection is highly improbable is one that cannot be answered *a priori*. Experience has shown, however, that the assumption that a Schick-negative person is, for all practical purposes, immune, is pragmatically sound.

Prophylactic Immunization.—It was early observed that experimental animals can be immunized to diphtheria by the injection of living cultures of the bacilli after a protective dose of antitoxic serum or by the inoculation of toxin neutralized with antitoxin. Theobald Smith[41] suggested the use of toxin-antitoxin mixtures in the immunization of horses, and the same method was used by von Behring[42] a few years later to immunize children. The use of toxin-antitoxin for the immunization of man, however, was developed largely through the efforts of Park in New York City from 1913 onwards.

Toxin-Antitoxin.—The mixture usually used contains 0.1 L_+ dose of toxin per cubic centimeter. The toxin is slightly underneutralized (5 cc. of the mixture should produce diphtheritic paralysis in 300 gm. guinea pigs) but depends for its immunizing efficiency not on the slight excess of toxin but on a slow dissociation of the toxin-antitoxin complex to liberate free toxin. Administered in 3 doses of 1 cc. each at intervals of one to two weeks, toxin-antitoxin produces an immunity in 85 per cent of individuals inoculated.[43] The immunity develops slowly, and one to six months may be required for the Schick reaction to become negative. Accidents may occur as a consequence of dissociation of the toxin-antitoxin mixture—freezing in one instance produced such dissociation—but these are rare, particularly with the 0.1 L_+ dose mixture. There is, of course, the possibility of sensitization of the inoculated individual to horse serum.

Toxoid.—The use of formol toxoid or anatoxin as an immunizing agent was introduced by Ramon in 1923 and has been widely adopted. As pointed out elsewhere (p. 255), toxin treated with formaldehyde (in this case a potent toxin of more than 15 Lf doses per cubic centimeter is incubated with 0.3 to 0.4 per cent formalin at 37° C. for one month) loses its toxicity but retains its antigenicity and is a highly efficient immunizing agent. The administration of this material in three doses of 0.5, 1.0 and 1.0 cc. at intervals of two to three weeks renders 95 per cent of persons Schick-negative.[44] It was at first thought that toxoid might entirely replace toxin-antitoxin as an immunizing agent, but this has not proved to be the case. Reactions to the bacillary protein, while not of great importance as a rule in young children, may be relatively severe in older persons, and its use

[40] *Cf.* Reh. Schweiz. med. Wchnschr., 1938, *68*:1173; see also Grozin: Amer. Jour. Dis. Children, 1939, *57*:564; Caussade: Rev. Med. de Nancy, 1939, *67*:321.

[41] Smith, T.: Jour. Med. Res., 1907, *16*:359; Jour. Exp. Med., 1909, *11*:241.

[42] von Behring: Deut. med. Wchnschr., 1913, *39*:873.

[43] *Cf.* Park: Jour. Amer. Med. Assn., 1922, *79*:1584.

[44] For a detailed discussion of diphtheria toxoid see Ramon: Ann. de Med., 1937, *42*:314; Ann. Inst. Pasteur, 1939, *62*:5.

is best restricted to children under twelve years of age. Reactivity may be tested for by the intradermal injection of toxoid—the *Moloney test.*

Toxoid-antitoxin floccules (the precipitate coming down at the optimal antigen-antibody ratio) have been used in England to a considerable extent. There is, presumably, a partial purification of the toxoid by precipitation with antibody.[45] This material and toxin-antitoxin floccules have not been widely used in the United States.

Alum-Precipitated Toxoid.—It has been found[46] that toxoid precipitated with potassium alum (small amounts, 1 to 2 per cent, are required) is superior as an immunizing agent to ordinary formol toxoid. Present preparations are treated with charcoal prior to alum precipitation to remove color and extraneous nitrogenous material.[47] The precipitate is insoluble (it may be redissolved in sodium citrate or sodium tartrate) and remains in the subcutaneous tissue for a considerable period of time, thus providing a prolonged antigenic stimulus. It was first thought that a single injection of this material was sufficient to provide a solid immunity; some of the early reports indicated that 90 to 95 per cent of Schick-positives became Schick-negative as a consequence of a single injection. The administrative advantages of a single injection are, of course, obvious. It has become increasingly clear in recent years, however, that a single injection is not sufficient; as little as 11 per cent conversion has been reported. There is also a tendency for such Schick-negatives to revert to Schick-positives within a year or two, though why this should be the case is not at all clear. It seems established that a single injection is not sufficient but two injections provide a solid immunity. The primary dose should be not less than half the total toxoid given and may be as great as two-thirds of it.[48] Alum toxoid has the same tendencies to produce untoward reactions in older persons that are observed with formol toxoid.

Passive Immunity.—Susceptible, *i.e.*, Schick-positive, individuals may be passively immunized to diphtheria by the injection of antitoxic horse serum or purified preparations of antitoxin. Such immunity is of relatively short duration and is not effective for longer than two or three weeks at the most. Passive immunization is not so extensively practiced now as it formerly was but is, of course, indicated in the case of susceptible individuals who are directly exposed to the disease. Except on a small scale, as in a hospital ward, it is not practical to control epidemic diphtheria through passive immunization.

The use of combined active-passive immunization in which both toxoid and antitoxin are given simultaneously, the former in protective amounts, has been of some interest. More recent work indicates that the passive protection conferred by antitoxin does not interfere seriously with the immune response though there is a period of low immunity, after the second

[45] For a discussion of this material see Watson, Taggart and Shaw: Jour. Path. Bact., 1941, *53*:63.

[46] Glenny, Pope, Waddington and Wallace: Jour. Path. Bact., 1926, *29*:38.

[47] For details of preparation see Barr, Pope, Glenny and Linggood: Lancet, 1941, *ii*:301.

[48] *Cf.* Bousfield: Brit. Med. Jour., 1943, p. 706.

or third week, after the passive protection has been exhausted. A second inoculation of toxoid is, of course, highly desirable.[49]

The Therapeutic Use of Antitoxin.—Serum therapy in diphtheria is more successful than in any other disease, and there is no question of its efficacy in reducing the case-fatality rates. As in the case of tetanus and botulism, the therapeutic administration of antitoxin cannot bring about repair of tissues already damaged by toxin. Early administration is, therefore, essential, and there is progressive increase in the case-fatality rate with each day's delay. Park advises in mild cases 3000 to 5000 units, in moderately severe cases 10,000 units, and in severe toxic cases 20,000 units or more in adults and 10,000 to 20,000 in children. There is no limit, beyond the volume, to the number of units that may be safely injected. Antitoxin is generally administered intramuscularly but in severe cases may be given intravenously. It is completely ineffective when given by mouth.

Of considerable practical significance is the concentration of antitoxin, since the volume injected is a limiting factor. Usually horse serum contains

SUSCEPTIBILITY OF VARIOUS AGES TO DIPHTHERIA

(As Indicated by the Schick Test)

Age	Susceptible, Per Cent
Under three months	15
Three to six months	30
Six months to one year	60
One to two years	70
Two to three years	60
Three to five years	40
Five to ten years	30
Ten to twenty years	20
Over twenty years	12

500 to 700 units per cc. and exceptionally 1000 to 1500. Concentration of the antitoxin by salting out and other procedures is generally practiced, for, although some antitoxin is lost in the process, the concentration is increased with a corresponding reduction in the volume to be injected.

Epidemiology.[50]—The epidemiology of diphtheria is considerably better understood than that of any other disease, in part because the causative agent can be isolated with relative facility from infected individuals, and in part because the Schick test allows the differentiation of the immunes and the non-immunes. As in the case of other respiratory diseases, infectious material leaves the body in the secretions of the nose and throat, is transmitted from man to man by contact or infective droplets, and enters the body via the mouth and nose. Furthermore, the diphtheria bacillus is disseminated not only by persons with the disease but also through the agency of healthy carriers in whom there is no clinical evidence of infection. Unlike

[49] See Downie, Glenny, Parish, Smith and Wilson: Brit. Med. Jour., 1941, p. 717; Phair and Root: Amer. Jour. Hyg., 1942, *35*:377.

[50] For a critical discussion of the epidemiology of diphtheria during the past forty years see Russell: Med. Res. Council (Great Britain), Spec. Rept. Ser. No. 247, 1943.

many of the diseases of the respiratory tract, however, diphtheria is an immunizing disease and prolonged or repeated contact with the bacillus frequently results in the development of a solid immunity to the disease in its clinical manifestations.

Immunity and Susceptibility.—Schick testing indicates that while susceptibility is low in the first six months of life, the proportion of Schick-positives increases rapidly and is at a maximum in children under four or five years of age, then gradually declines until 80 per cent or thereabouts of adults are Schick-negative. (See the accompanying table.) The initial immunity of the very young is passively transferred from the mother and is not of long duration. The increase in the proportion of immunes, however, is by no means entirely a result of recovery from clinical diphtheria, and the question arises as to how these individuals acquire an active immunity.

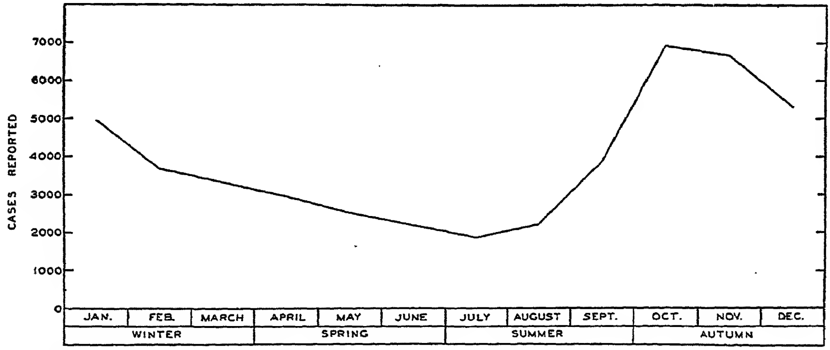


Fig. 133.—The seasonal incidence of diphtheria. Averages of reported cases by months for the years 1930 to 1938 inclusive. Data from Supplements to Public Health Reports.

Carriers.—As indicated above, healthy individuals may harbor virulent diphtheria bacilli in their throats. These carriers need be neither immunes nor convalescents and are, for the most part, casual carriers. There is no precise information concerning the duration of this transient carrier state; it may possibly be about two weeks. The proportion of carriers has been investigated by a number of workers. In a study of Baltimore school children, Doull and Fales[51] found an average carrier rate of 2.32 per cent from November to May. On the basis of this Frost,[52] has estimated the carrier incidence in the five to fourteen age group in that city to be 2538 per 10,000. At this rate 75 per cent of the population becomes infected at least once in five years, 95 per cent in ten years, and over 99 per cent in fifteen years, while very considerable proportions would suffer repeated infections, the average being 2.5 infections per person in ten years. Others have recorded considerably higher carrier rates; Dudley[53] has reported 6.6 per cent in a

[51] Doull and Fales: Amer. Jour. Hyg., 1923, *3*:604.
[52] Frost: Jour. Prev. Med., 1928, *2*:325.
[53] Dudley: Jour. Hyg., 1932, *32*:193.

boys' school, and repeated swabbings showed that at least 40 per cent carried the diphtheria bacillus at one time or another during the yearly period.

There is, it appears, ample opportunity for contact with virulent diphtheria bacilli, and there is every reason to suppose that the increasing proportion of Schick-negatives in the progressively higher age groups is a consequence of an active immune response to the presence of these microorganisms in the nose and throat. It may be noted parenthetically that a similar situation may very likely prevail in certain other diseases in which technical difficulties have prevented its demonstration.

It may be asked why contact with virulent diphtheria bacilli does not result in clinically apparent infection more often. The production of disease is, of course, dependent upon the balance between virulence and

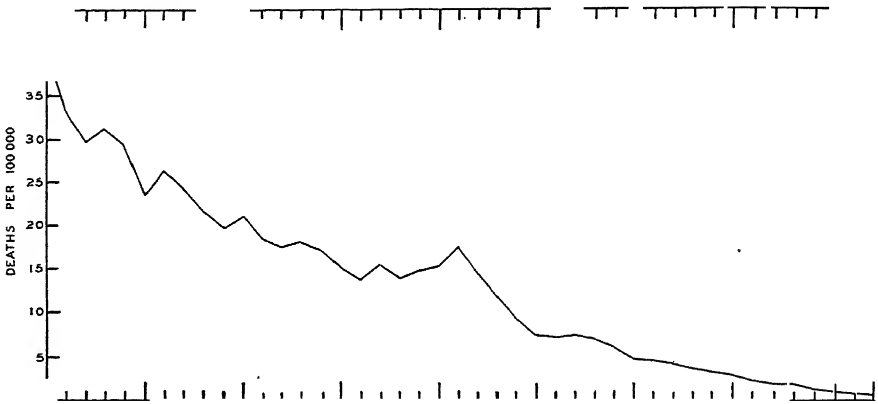


Fig. 134.—The prevalence of diphtheria in the Registration Area since 1900 as indicated by the uncorrected death rates. From data compiled by the Bureau of the Census.

resistance, as pointed out in an earlier chapter (Chap. 8). Climate appears to be of some importance, for the proportion of Schick-negatives in tropical countries is quite as high as in the temperate zones yet clinical diphtheria is much less common. Racial factors may be involved also, for in the Baltimore studies the carrier rate in Negroes was not significantly different from that in whites, yet the morbidity rate for the former was much lower.

The opportunities for the transmission of infection from person to person are reflected in both the morbidity and the mortality rates. There appears, for example, to be a direct relation between school attendance and the incidence of diphtheria, and the deaths from this disease tend to be concentrated in the preschool age group with increasing urbanization.

The Control of Diphtheria.—It will be obvious from the above considerations that diphtheria is widely disseminated in the human population and cannot be controlled by the isolation of carriers or, except in a strictly limited sense, by quarantine of cases. The control of diphtheria

is entirely a matter of immunization and, if a sufficiently large proportion of the susceptible population is rendered immune, the prevalence of clinical diphtheria should decrease. Godfrey[54] has found that the immunization of 50 per cent or more of the children of school age, five to fourteen years of age, did not produce a fall in the incidence of diphtheria in a number of large American cities, but that when 30 per cent or over of the preschool children were immunized there was a definite reduction in the incidence of diphtheria not only among these children but in the community as a whole. Immunization confers a marked, though not absolute, protection in the individual case, of course, and it would appear best from both the individual and group points of view that immunization be effected early.

To what extent prophylactic inoculation and the therapeutic use of antitoxin have influenced the decline in diphtheria shown in Fig. 134 is problematical. The disease was endemic during the first half of the nineteenth century though showing increasing epidemic tendencies. Between 1850 and 1860 a great pandemic developed, apparently from a focus in France, which swept over the world. A high mortality was maintained for twenty-five or thirty years, then around 1885 a decline set in which has continued to the present. Immunization was not generally practiced until about 1920, though antitoxin therapy began somewhat earlier. Russell[50] is of the opinion that the decline in certain areas, such as New York, in which active immunization has been extensively applied is more rapid than could be expected from the trend of pre-immunization years, and attributes this increase to prophylactic inoculation. In general, however, the trend does not appear to be markedly affected. The decline in diphtheria mortality in the large cities of the United States has proceeded rapidly and the rates are considerably less than for the country as a whole; since 1939 the rate has been less than 1.0 per 100,000, the 1942 rate was somewhat higher, 0.70, than that of 1941, 0.56, and the rise is attributed to increased travel in part.[55]

THE DIPHTHEROID BACILLI

Bacilli morphologically similar to the diphtheria bacillus, but which do not produce toxin and are apparently quite harmless, are frequently observed in the throat. These bacilli were observed by Löffler and by von Hofmann-Wellenhof[56] and are generally designated as *Hofmann's bacillus*, *C. hofmannii*, *C. pseudodiphtheriae* (Bergey) or the pseudodiphtheria bacilli. These microorganisms differ from the diphtheria bacillus in that they are somewhat shorter and plumper, do not show the presence of metachromatic granules, and do not ferment dextrose. They are non-pathogenic for experimental animals.

Diphtheroid bacilli isolated from a form of conjunctivitis known as xerosis have been termed *C. xerose* and have been supposed by some to be causally associated with this affection. It is probable, however, that this bacterium is present as contamination and is not the cause of this disease.

[54] Godfrey: Amer. Jour. Pub. Health, 1932, *22*:237.
[55] Jour. Amer. Med. Assn., 1943, *122*:1079.
[56] von Hofmann-Wellenhof: Wien. med. Wchnschr., 1888, *38*:65, 108.

It is thought by some workers to be identical with Hofmann's bacillus. Another diphtheroid bacillus, *C. acne*, has been isolated from acne pustules, but here again there is no evidence to support the assumption of a causal relation. *C. acne* stands apart from the other bacilli of this group in that it is microaerophilic and grows profusely under anaerobic conditions, with the formation of a pink pigment.

Other diphtheroid bacilli have been found associated with diseases of lower animals. *C. pyogenes* is associated, probably causally, with a variety of suppurative infections of domestic animals, including cattle, sheep, goats and pigs. It produces a weak soluble toxin immunologically distinct from diphtheria toxin.[57] Another diphtheroid bacillus, *C. ovis*, or the Preisz-Nocard bacillus, is pathogenic for sheep and horses, in which animals it produces lesion of "pseudotuberculosis," and forms a weak soluble toxin

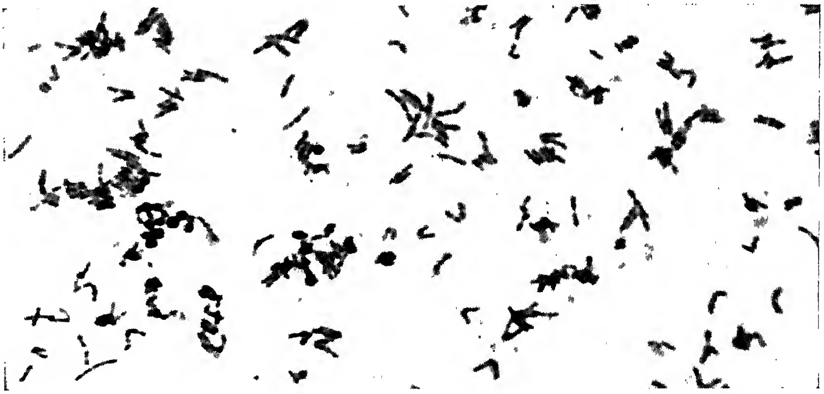


Fig. 135.—*Corynebacterium hofmannii*; smear from pure culture stained with alkaline methylene blue. Note the irregular staining, club-shaped forms, and general close resemblance to *C. diphtheriae*. × 1050.

distinct from diphtheria toxin. Still another species, *C. murisepticum*, produces a septicemia in mice and is apparently pathogenic for no other animals.

A variety of other diphtheroid bacilli have been described, none of which is of any pathological significance. The diphtheroid bacilli found in the human throat, however, may be confused with the diphtheria bacillus and hence assume some significance in this connection. As indicated above, granular forms do not occur, or at best only infrequently; these bacilli are pleomorphic but not to the same degree as the diphtheria bacillus. No absolute distinction can be made on morphological grounds, however, and the diphtheria bacillus must be identified by the virulence test, the formation of diphtheria toxin specifically neutralizable by diphtheria antitoxin.

[57] This bacillus has recently been thoroughly studied by Lovell: Jour. Path. Bact., 1937, *45*:339; *ibid.*, 1939, *49*:329; *ibid.*, 1941, *52*:295.

CHAPTER 31

MYCOBACTERIUM

Order: *Actinomycetales.* Family: *Mycobacteriaceae.* Genus: *Mycobacterium.* Slender rods, straight or curving, sometimes irregular in form with slight or occasional branching. Stain with difficulty but resist decolorization with mineral acids, *i.e.*, are acid-fast. Gram-positive, non-motile, non-spore-forming, aerobic. Several species are pathogenic for animals. Type species: *Mycobacterium tuberculosis.*

This genus includes a number of species of related bacteria which are most conveniently considered in three groups. The first includes the mammalian tubercle bacilli, *Mycobacterium tuberculosis* var. *hominis* and *Mycobacterium tuberculosis* var. *bovis*, and the avian tubercle bacillus, *Mycobacterium avium.* In the second group there are Hansen's bacillus or *Mycobacterium leprae*, and the rat leprosy bacillus, *Mycobacterium leprae murium.* The third group is made up of Johne's bacillus, or *Mycobacterium paratuberculosis*, and certain acid-fast bacilli isolated from cold-blooded animals, together with the saprophytic acid-fast forms.

THE TUBERCLE BACILLI

Tuberculosis is an old disease of man and is still one of the most widespread; about 75,000 persons die of tuberculosis in the United States each year. Its infectious nature was suspected by Fracastorius in the early part of the sixteenth century, and Villemin[1] showed, in 1865, that the disease could be transmitted by the inoculation of tuberculous material. It was in 1882 that Koch[2] demonstrated the tubercle bacillus by special staining methods, isolated and grew it in pure culture, and reproduced the disease by the inoculation of the bacilli.

Morphology and Staining.—The tubercle bacilli are slender, sometimes slightly curved rods 2 to 4 μ in length and 0.3 to 1.5 μ in breadth. They occur singly but are often found in small groups, sometimes in compact masses in which the individual bacilli cannot be distinguished. The bacilli of the human variety tend to be somewhat longer and more slender than those of the bovine type, but the morphology of both is variable and no distinction can be made on this basis. The bacillary form is generally retained in the tissues; in culture longer filamentous forms are sometimes seen together with swollen or club-shaped cells resembling the diphtheria bacillus. Branched forms are present in cultures of the avian tubercle bacillus but are rarely seen in cultures of the mammalian bacilli. The occurrence of filamentous forms and true branching indicates the close relation of these bacilli to the higher fungi; hence the name *Myco-*

[1] Villemin: Gaz. Hebdom., 1865, 2 s., p. 5.
[2] Koch: Ber. klin. Wchnschr., 1882, *19*:221.

bacterium and the placing of these microorganisms, together with the diphtheria bacillus, in the order *Actinomycetales*.

The tubercle bacillus is non-motile and non-spore-forming and produces a capsular substance in artificial cultures, particularly when grown upon serum media. The granular structure of the individual cells is marked and particularly well shown in the electron-microscope photomicrograph on p. 39. Vacuoles often occur in abundance and may even give the stained cell the appearance of a chain of cocci. The significance of the small, deeply staining bodies sometimes observed within the cells is not clear; they do not exhibit the enhanced resistance characteristic of spores.

The tubercle bacilli cannot be stained by the usual staining methods that are effective with other bacteria, for there is a marked resistance to the penetration of dyes into the cell that is associated with the presence

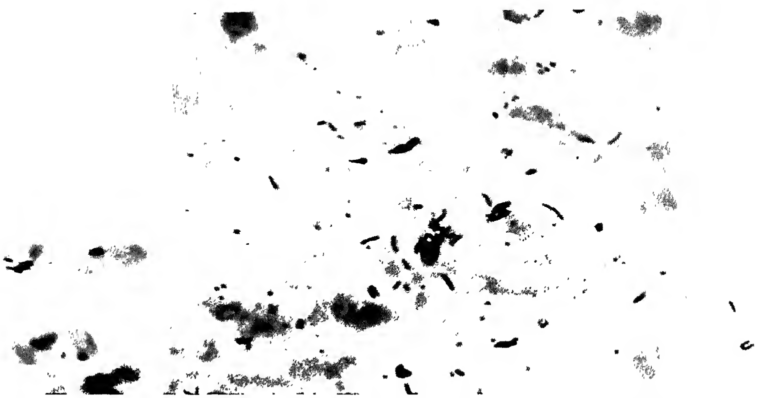


Fig. 136.—*Mycobacterium tuberculosis*. Acid-fast stained smear of tuberculous sputum. × 1050.

of relatively large amounts of unsaponifiable wax. The cells may, however, be stained in two or three minutes by steaming carbol fuchsin or by prolonged (twenty-four to thirty-six hours) exposure to the dye at room temperature. Once stained, the bacilli are difficult to decolorize and resist the action of alcohol and dilute solutions of mineral acids and for that reason are termed "acid-fast." They may be demonstrated in smears by the Ziehl-Neelsen method, in which the smear is stained with hot carbol fuchsin, decolorized with dilute acid, and counterstained with a dye of contrasting color. Methylene blue is most commonly used but some workers prefer other stains such as picric acid, Bismarck brown, etc.[3] Non-acid-fast bacilli may be observed in young cultures.

More recently fluorescent microscopy has been applied in the detection of tubercle bacilli in sputum smears, concentrates and similar material. The smear is stained with carbol-auramine, a solution of auramine in 3 per cent phenol, decolorized with acid alcohol, and examined in ultraviolet

[3] *Cf.* Pottenger: Amer. Rev. Tuberc., 1942, *45*:558.

light. There is no counterstain. The ultraviolet irradiation need not be intense and a brilliant filament lamp with a blue ultraviolet-transmitting filter and an aluminum mirror suffice for use with the ordinary microscope.[4] The tubercle bacilli retain the dye which fluoresces in ultraviolet light, and appear as brilliant yellow bacilli on a dark background. The use of cresol in concentration of the bacilli interferes with the examination, for it fluoresces also. This staining method may also be used for tissue sections.[5] Auramine is retained by the tubercle bacillus in the same way that fuchsin is retained, *i.e.*, by virtue of the presence of the acid-fast wax mycolic acid, and bacilli rendered non-acid-fast by treatment with organic solvents no longer retain auramine.[6] Three to four times as many tubercle bacilli may be found in a given smear by fluorescent microscopy as by examination of the conventional Ziehl-Neelsen stains, and this is presumably due to the greater retention of auramine.[7]

The tubercle bacilli are gram-positive. Aniline gentian violet must be applied warm for two or three minutes. It has been suggested that the iodine solution plays no part in the retention of the stain as in the case of other gram-positive bacteria and the failure to decolorize is a consequence of the acid-fast nature of the cells.[8]

Non-acid-fast but gram-positive granules, known as *Much granules*, were described by Much[9] as occurring in the material from cold abscesses and elsewhere in which acid-fast bacilli could not be demonstrated but which, nevertheless, proved to be infective. Considerable numbers of acid-fast bacilli, perhaps 100,000 per cubic centimeter, must be present, however, before there is a reasonable chance of finding them in smears. Much maintained that these granules are viable and virulent and give rise to typical acid-fast rods. They have been observed by others but their significance is open to question. Some workers regard them as degeneration products.

In broth cultures there is a thick, wrinkled skin of surface growth which tends to spread up the sides of the flask; masses of bacilli may become detached and sink to the bottom as a lumpy sediment. Growth on the surface of semisolid media is generally dry and granular with nodular, heaped-up areas. The human variety of the tubercle bacillus usually produces a pale yellow or orange-yellow growth on serum-containing media and a creamy or white growth in the absence of serum. The bovine variety is not pigmented on serum media. Some avian strains give a faint pink-colored growth on egg media. A peculiar almond-like odor is often noticeable in cultures of these bacteria.

Physiology.—The tubercle bacillus is an obligate aerobe and will not grow under completely anaerobic conditions. The mammalian varieties

[4] For detailed discussions of the method and its application see Richards, Kline and Leach: Amer. Rev. Tuberc., 1941, *44*:255; Bogen: *ibid.*, 1941, *44*:267; Lind and Shaughnessy: Jour. Lab. Clin. Med., 1942, *27*:531.

[5] *Cf.* Crossman and Lowenstein: Jour. Lab. Clin. Med., 1943, *28*:1349.

[6] Bekker and Tasman: Arch. f. Hyg. u. Bakt., 1941, *127*:7.

[7] Richards: Science, 1941, *93*:190.

[8] Kretschmer: Jour. Lab. Clin. Med., 1934, *19*:350.

[9] Much: Beitr. klin. Tuberk., 1907, *8*:85.

grow best at 37° C. and not at all below 30° C. or above 42° C.; the optimum temperature for the avian type, however, is 40° C. Growth is relatively slow, and four to six weeks are generally required for an abundant growth, although minute colonies appear in eight to ten days. Most strains of the avian type adapt themselves readily to culture on artificial media and in time are able to grow much more rapidly, but others remain slow-growing.

The tubercle bacillus is difficult to cultivate upon primary isolation. Blood serum, coagulated by inspissation, was used by Koch, and this medium still remains one of the most satisfactory. Media containing eggs, either the yolk or yolk and white combined, glycerol, and sometimes dyes, are also used for primary isolation. Dorset's egg medium is simply whole egg mixed with a little water and coagulated in slant form by inspissation. Petroff's medium, one which is widely used, consists of an infusion base to which eggs, glycerol and gentian violet have been added, and is steril-

Fig. 137.—Colonies of the human variety of the tubercle bacillus, H–37 strain, on Lowenstein's medium, five weeks' incubation. × 3.

ized by inspissation. Lowenstein's medium is more complex and contains egg, potato meal, bone marrow infusion, citrate, glycerol and asparagin. Glycerolated potato is used by the French workers. Corper's medium is glycerolated potato with the modification that the pieces of potato are soaked for a short time in a solution of crystal violet before sterilization with the glycerol solution. The dye in this and Petroff's medium serves to inhibit the growth of contaminating bacteria.

The human variety of the tubercle bacillus grows more abundantly on all of these media than does the bovine variety, and for that reason it is termed "eugonic" and the bovine type "dysgonic." These two varieties also differ in that glycerol is markedly favorable to the growth of the human type but does not so affect the bovine type. It is not known why glycerol exerts this favorable effect; attempts to substitute related compounds such as isopropyl alcohol, propyl alcohol, glycol, trimethylene glycol and inositol have not been successful.[10] Glucose, however, acts in

[10] Fernbach and Rullier: Compt. Rend. Soc. Biol., 1939, *131*:1142.

much the same manner as glycerol. Egg-yolk has been reported[11] to contain a lipoid growth factor, but this appears to stimulate rather than be essential to growth. Thiamin, pyridoxin and riboflavin do not stimulate growth.[12]

Growth occurs much more readily and upon simpler media after primary isolation. The human tubercle bacillus grows well upon nutrient agar or broth containing glycerol (2 to 5 per cent) and has been cultivated in a variety of synthetic solutions. One of the best known of these is Long's synthetic medium which contains glycerol, asparagin, citrate and inorganic salts. The bovine type grows poorly or not at all on these media and is benefited only slightly by the presence of glycerol. The avian type of tubercle bacillus, however, grows better than the human type after a few transfers and good growth may be obtained on nutrient agar in the absence of glycerol. Like the human type, however, the avian bacillus grows much more profusely in the presence of glycerol. These cultural differences in the three types of tubercle bacilli are of little significance in their early differentiation, for several transfers over a period of months are necessary before they are obvious. They are summarized in the table on p. 586.

The biochemical reactions of the tubercle bacilli have not been studied at length. Growth occurs in milk, but no visible change is produced. Indol is said not to be formed. No acidity is developed in sugar broths, but it has been found by analytical methods that dextrose, arabinose and sometimes sucrose are utilized while lactose is not.[13] Whether the oxidation proceeds to completion or whether formed acids are neutralized by liberated ammonia is not known. In glycerol broth, cultures of the bovine type become alkaline while those of the human type become slightly acid. It has been reported[14] that the source of alkali is the nitrogenous constituents of the medium, and acid is produced from glycerol but is further oxidized. All intermediate gradations occur, however, and no differentiation between the two types is possible on this basis.

Chemical Composition.—The chemical composition of the tubercle bacilli has been more intensively investigated than that of any other bacteria. These bacilli are of particular interest in this connection because of their high content of lipoidal substances which may make up as much as 40 per cent of the dry weight. Protein, a considerable proportion of which is nucleoprotein, makes up about half the dry weight and polysaccharides are found in relatively small amount.[15]

The lipoids have been studied at length by Anderson[16] and his coworkers. In addition to neutral fat, two general types of material may be distinguished:

(*a*) Phospholipoid, containing saturated and unsaturated fatty acids

[11] Boissevain and Schultz: Amer. Rev. Tuberc., 1938, *38*:624.

[12] Heise and Steenken: Amer. Rev. Tuberc., 1941, *44*:635.

[13] Merrill: Jour. Bact., 1930, *20*:235.

[14] Henley and LeDuc: Amer. Rev. Tuberc., 1939, *40*:313.

[15] See the review of the protein constituents of the tubercle bacillus by Seibert: Bact. Rev., 1941, *5*:69.

[16] *Cf.* Anderson: Physiol. Rev., 1932, *12*:166; Chem. Rev., 1941, *29*:225.

including the well-known palmitic, linoleic and linolenic acids, together with two acids peculiar to the tubercle bacillus, *phthoic acid*, isomeric with cerotic acid, and *tuberculostearic acid*, isomeric with stearic acid and optically inactive.

(*b*) An acid-fast wax, containing polysaccharides hydrolyzing to mannose, arabinose and galactose; a soft wax which is a complex glyceride; and an unsaponifiable wax (acid-fast) made up of higher alcohols and including a high molecular weight saturated hydroxymethoxy acid termed *mycolic acid*[17] and a higher alcohol designated *phthiocerol*.

Some of these substances appear to be physiologically active. The unsaponifiable acid-fast wax apparently stimulates the multiplication of undifferentiated connective tissue cells, and phthoic acid induces a proliferation of epithelioid cells.[18] Whether they are immunologically active is not clear. It may be noted that a yellow pigment is found in the neutral fat which is designated *phthiocol* and is a hydroxynaphthaquinone which may be reversibly reduced at a relatively low potential.[19]

Polysaccharide mixtures, containing immunologically active and inactive substances, have been isolated from mammalian tubercle bacilli[20] but their significance is not as yet understood. The protein constituents of the cell appear to be the most important immunologically and have been studied in connection with the preparation and activity of the various tuberculins which are considered in a later section.

Resistance.—Although exhibiting much the same degree of resistance to heat as the vegetative cells of other bacteria, the tubercle bacilli are relatively highly resistant to drying, chemical disinfectants and other deleterious environmental influences, very likely as a consequence of their content of wax. In putrefying sputum the bacilli may remain viable for weeks or months and in dried sputum kept in a cool dark place for as long as six to eight months. Sputum that is completely dried, so that particles are capable of floating as dust in the air, may be infective for eight to ten days.[21] In dried sputum they may survive 100° C. for an hour but are killed in the usual way by moist heat. Phenol penetrates the bacilli only slowly and a 5 per cent solution requires twenty-four hours to kill the bacilli in sputum. The action of other disinfectants is similarly retarded, and it may be noted that the hypochlorites have almost no effect on these bacteria. Tubercle bacilli, however, are readily killed by exposure to direct sunlight; bacilli from cultures are killed within two hours but in sputum may survive twenty to thirty hours of such direct exposure.

Variation.—The variability of the tubercle bacilli has been studied extensively, particularly in recent years, but with inconclusive results. Colonial variants, thought by some to be analogous to the S and R variants of other bacteria, have been observed, and it has been claimed by some workers that the S type is the more virulent, and by others that

[17] *Cf.* Stodola, Lesuk and Anderson: Jour. Biol. Chem., 1938, *126*:505; Lesuk and Anderson: Jour. Biol. Chem., 1940, *136*:603.

[18] *Cf.* Sabin: Physiol. Rev., 1932, *12*:141; Amer. Rev. Tuberc., 1941, *44*:415.

[19] Ball: Jour. Biol. Chem., 1934, *106*:515.

[20] See Menzel and Heidelberger: Jour. Biol. Chem., 1939, *127*:221.

[21] For example, see Smith: Amer. Rev. Tuberc., 1942, *45*:334.

virulence and colonial morphology are independent. It may be noted that the colonial morphology of the tubercle bacilli is, to a considerable degree, a transient adaptation to environmental conditions and prompt alteration of colonial appearance results on transfer to a different medium. For example, Steenken[22] has observed that colonies growing in the presence of ether extract of egg yolk are smooth and markedly different from the usual colonial type, but the effect is only a temporary physical one. The status of the S-R variation in the tubercle bacilli is, then, by no means clear as yet.

Bacille Calmette-Guérin.—A bovine strain of the tubercle bacillus was rendered completely avirulent by Calmette,[23] who cultivated it over a long period of time (230 transfers in thirteen years) on bile-glycerol-potato medium. This strain is designated *B.C.G.* (*Bacille Calmette-Guérin*) and has been of particular interest in connection with active immunization against tuberculosis. The nature of the change which resulted in the loss of virulence is completely unknown. The loss appears to be permanent and virulence does not reappear on transfer to ordinary media; Petroff,[24] however, has separated "rough" and "smooth" variants of *B.C.G.*, and the "smooth" type proved virulent for guinea pigs.

Life Cycles.—The pleomorphic tendencies of the tubercle bacilli, coupled with the occurrence of non-acid-fast rods in young cultures and the granular elements described by Much, have been interpreted by a number of workers as indicative of a cyclic succession of morphologic types, or life cycle, through which these bacilli go.[25] As pointed out in an earlier chapter (Chap. 6), the evidence for such developmental cycles is not definitive but rather a matter of interpretation. Whether swollen, misshapen cells, filamentous or branching forms and granular elements, such as those described by Much, are to be regarded as sexually reproducing forms, gonidia and the like, or as products of degenerative changes is as yet purely a matter of opinion. It may be noted that the objective evidence of micromotion pictures does not support the assumption of a complex life cycle in the development of the tubercle bacilli.[26]

Filterable Forms.—Associated by some with the concept of life cycles is that of the existence of tubercle bacilli in a filterable form or "ultravirus." There seems to be little doubt that some cultures or preparations of tubercle bacilli contain forms that may pass diatomaceous earth or unglazed porcelain filters, but whether these are dwarfed bacilli or fragments of bacilli with the power to regenerate, or whether they represent a distinct "phase" in the life history of the microorganisms has yet to be determined.

Pathogenicity for Man.—In the United States in 1940 there were 60,428 deaths from tuberculosis reported, 55,576 from respiratory tuberculosis, non-respiratory infections accounting for a little over 8.3 per cent

[22] Steenken: Amer. Rev. Tuberc., 1940, *42*:422.

[23] *Cf.* Calmette: *Tubercle Bacillus Infection and Tuberculosis in Man and Animals* (transl.). Williams & Wilkins Company. Baltimore. 1923.

[24] Petroff: Proc. Soc. Exp. Biol. Med., 1927, *24*:632; Petroff, Branch and Steenken: *ibid.*, 1927, *25*:14.

[25] *Cf.* Sweany: Amer. Rev. Tuberc., 1928, *17*:63; Kahn: Tubercle, 1930, *11*:202.

[26] Wyckoff: Amer. Rev. Tuberc., 1934, *29*:389.

of the deaths. Tuberculosis is among the first three leading causes of death in a relatively large portion of the life span (15 to 49) and holds first place in the 15–34 age group, second in the 35–39 age group, and third in the 40–49 group. In 1939–1941 it accounted for 10.4 per cent of the white male, 22.9 per cent of the white female, 28.8 per cent of the non-white male and 37.8 per cent of the non-white female deaths.[27] Tuberculosis is clearly one of the great killing diseases of mankind.

The mammalian tubercle bacilli, both bovine and human varieties, are pathogenic for man. The human type is practically always responsible for pulmonary tuberculosis in adults and is usually found in children also. The bovine variety may occur occasionally in pulmonary tuberculosis in children but is more often found in infections of other tissues. More than half of the cases of cervical adenitis and abdominal tuberculosis in children are infections with the bovine tubercle bacillus, and it has been estimated (1910) that this type was responsible for 6 to 10 per cent of the deaths from tuberculosis in young children; the percentage is probably lower now as a result of decreased incidence of tuberculosis in cattle in this country. Bovine tuberculosis is, then, a slight, possibly a negligible, factor in adults but is a serious matter in children under five years of age. Mixed infections with the two types of tubercle bacilli have been reported but are rare.

The avian tubercle bacillus is, for all practical purposes, non-pathogenic for man, although a few instances of human infection have been reported. It has been supposed by some that Hodgkin's disease, a granulomatous inflammation of the lymphadenoid tissues of the body, is an infection with avian tubercle bacilli, but this appears not to be true.

Routes of Infection.—The tubercle bacillus may enter the body by way of the genito-urinary tract, the conjunctiva, the skin, the alimentary tract and the respiratory tract. Primary infection of the genito-urinary tract is possible but, under natural conditions, rarely occurs. Infection through the conjunctiva takes place readily under experimental conditions; its frequency under natural conditions is not known, for the cervical lymph glands, where the infection would first appear, are readily infected by other channels. Infection through the skin is relatively rare; whether the bacilli can penetrate the intact skin is uncertain, but they may enter through abrasions or other traumatic injuries. Primary infection of the skin generally results in verruca tuberculosa (pathologist's wart) or lupus vulgaris.

Infection by the alimentary tract is a consequence of the ingestion of tubercle bacilli in infected food, most commonly milk, and occurs with considerable frequency in children. In the upper portion of the alimentary tract the bacilli enter the body tissues through the faucial, pharyngeal and lingual lymphoid follicles and first affect the upper cervical and retropharyngeal lymph glands. Tuberculous lesions of the tonsils, it may be noted, are not infrequent though rarely conspicuous. The stomach is rarely a portal of entry, but the bacilli penetrate the intestinal mucosa by way of Peyer's patches.

The respiratory tract is the most frequent and most important route of infection with the tubercle bacillus. The coarser infectious particles in

[27] Yerushalmy, Hilleboe and Palmer: Pub. Health Repts., 1943, *58*:1457.

inspired air are filtered out and deposited on the nasal, buccal and pharyngeal surfaces and the bacilli, upon penetration, set up focal infections in the local lymphatic tissue. Fine droplets or particles of dust, however, may, and frequently do, enter the lungs directly.

The Spread of Infection in the Body.—Tubercle bacilli are disseminated through the body from primary or secondary foci of infection by way of the lymph, the blood stream, or directly by extension along contiguous surfaces. Distribution via the lymphatics occurs more readily in children than in adults, and the bacilli may localize at almost any point but most commonly in the lymph nodes. Bacilli present in the thoracic duct may, of course, gain entrance to the blood stream. The blood stream may also be invaded directly through erosion of a vessel wall by a focus of infection. The bacilli are transported throughout the body by the blood and give rise to acute miliary or chronic disseminated tuberculosis. Spread by ex-

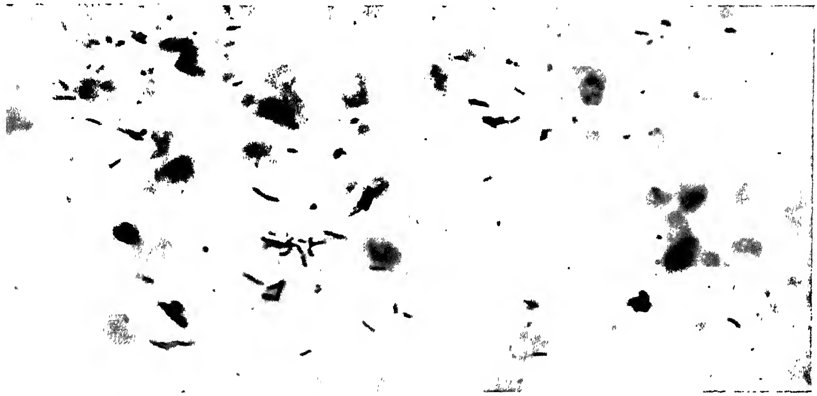


Fig. 138.—Tubercle bacillus. Acid-fast stained smear of pus from a liver abscess in a rhesus monkey. × 1050.

tension occurs most frequently in ulcerative pulmonary tuberculosis with the breaking down of foci of infection and the consequent presence of bacilli in the sputum. The pleura and pericardium may be invaded directly from a focus of infection in the lungs. The infection of such serous surfaces may be localized and fibrinous and result in adhesions, or may assume an acute miliary form. Direct extension may also occur elsewhere, as from the kidneys to the ureters and bladder, or into the peritoneal cavity and adjacent areas from intestinal ulcers.

It will be clear that practically every organ and tissue of the body may be invaded by the tubercle bacillus. Not all are invaded with equal frequency, however. Tuberculosis in man is most commonly the pulmonary form with a primary invasion of the apices, and over 90 per cent of the deaths from tuberculosis are due to the pulmonary type. Whether primary infection is a direct consequence of inhalation as believed by Koch, or whether it is in most cases of hematogenous origin following a prelim-

inary infection of the lymphatic system as urged by Calmette, is not altogether clear, but on the whole the evidence favors inhalation infection. Although pulmonary tuberculosis is by far the most common form of infection in adults, it is somewhat less frequent in children, but precisely how much less is not known with certainty. Pulmonary infection in children differs from that in adults in that the hilum glands are involved. Involvement of the lymphatics is frequent in children. The anatomical distribution of the lesions usually takes one or the other of two well-defined forms; in the one the lesions are found predominantly in the tracheobronchial lymphatics, and in the other in the mesenteric lymph nodes. In general, children show a tendency toward generalized infection.

Other tissues and organs are involved less frequently. The spleen, liver and kidneys are sometimes infected. Tuberculosis of the adrenals gives rise to Addison's disease. Infection of the skin or lupus is not uncommon. Bone and joint tuberculosis is more common in children than in adults, and tuberculous meningitis is not infrequent in the young. It will be clear that tuberculous infection may take a variety of clinical forms.

The Tubercle.—Lesions caused by the tubercle bacillus, in whatever part of the body they occur, usually possess a definite although not absolutely characteristic appearance and histological structure. Small nodules or tubercles, plainly visible to the naked eye, are so uniformly observed in all advanced infections with the tubercle bacillus that their presence has given the name to the disease. The young tubercle probably originates from the fixed cells surrounding the invading bacilli. By the proliferation of the fixed cells, elongated "epithelioid" cells are developed in more or less definite concentric layers and come to form the substance of the tubercle. So-called "giant cells" or "foreign-body giant cells" soon appear in the developing tubercle which are huge multinuclear masses of protoplasm thought by some to be especially distinctive of true tubercle formation, though it is doubtful if this criterion can be maintained. Either they are produced by the fusion of a number of macrophages or they are of single-cell origin. While the formation of epithelioid and giant cells is going on, leucocytes, at first polymorphonuclear leucocytes and later lymphocytes, cluster around the periphery of the tubercle. Degeneration of the tubercle eventually sets in, the central portion becomes necrotic, and this is followed by caseation and then by softening of the caseous mass.

In some cases calcium salts are deposited in the tubercle (calcification), converting it into a hard, dry, friable body which may become encapsulated and completely walled off from the surrounding tissues. In other instances, however, this healing process does not take place, and there is instead an extension with coalescence and the formation of confluent masses that may reach a diameter of 4 or 5 cm. The erosion of a blood vessel may occur, and the discharge of large numbers of the bacilli into the blood stream leads to a general diffusion of small tubercles of the size of millet seeds (acute miliary tuberculosis).

The early stages of tubercle formation, characterized by cell proliferation and leucocytic infiltration, are probably a response to a chemical or mechanical stimulus caused by the presence of the bacilli; the later changes,

leading to necrosis and caseation, may be attributed to the action of the bacterial products. The host becomes sensitized to the bacillary substance and the allergic response of the tissues is involved to no small extent.

Predisposing Factors.—Few diseases are as completely dominated by predisposing factors as tuberculosis. Infection with the tubercle bacillus is exceedingly common, and there are few adults, particularly those living in cities, who escape. The proportion of individuals coming to autopsy who show evidence of infection is very high and has been reported as high as 97 per cent. Of the more recent reports, for example, Sweany, Levinson and Stadnichenko[28] have found 25 per cent of ten-year-olds, 55 per cent of twenty-year-olds, 80 per cent of thirty-year-olds, and over 90 per cent of individuals over fifty years of age show calcified lesions of infection. The bacilli, then, are present in the great majority of adults and also in many children, but the development of clinical tuberculosis is restrained by the non-specific resistance of the host. Predisposing factors, therefore, are those which tend to interfere with normal physiological well-being and include such things as insufficient or unsuitable food, prolonged exposure to dampness, a sedentary life, chronic fatigue, etc. In the first World War the incidence of tuberculosis rose in this (see Fig. 141) and other countries, and in the second World War the same tendency was again apparent in England.[29] In both man and animals tuberculosis is a disease associated with confinement, of men in houses and cattle kept in stables. The opportunities for transmission of the infection are, of course, much greater in community life.

There is a marked occupational predisposition to tuberculosis in the dusty trades, and the constant inhalation of almost any kind of dust results in an increased incidence of pulmonary tuberculosis. Silica dusts, however, appear almost specifically to predispose to tuberculosis and the incidence of infection in those constantly exposed to such dusts is much higher than that in the general population.

It has long been suspected that there are familial or hereditary tendencies to tuberculosis in man. Resistance to infection in experimental animals is to some degree genetically determined, as pointed out elsewhere (p. 187), but the conclusive demonstration of a similar phenomenon in man is difficult owing to both a long generation time and the inability to carry out appropriate breeding experiments. The incidence of new infection in tuberculous families is, of course, considerably greater than in the general population, but whether this is a consequence of increased risk alone or in part of genetically determined factors is not clear. In any case the disease itself is not inherited and congenital tuberculosis rarely if ever occurs.

The relation of childhood infection to clinical tuberculosis in the adult has been a matter of considerable interest, and it has been suggested that pulmonary tuberculosis of the young adult may, in many instances, be a consequence of the lighting up of an old infection. The evidence, however, is against this view, and it is probable that tuberculosis in the adult is a consequence of reinfection rather than of a flaring up of the healed or par-

[28] Sweany, Levinson and Stadnichenko: Amer. Rev. Tuberc., 1943, *48*:131.

[29] Med. Res. Council (Great Britain), Spec. Rept. Ser., No. 246, 1942.

tially healed lesions from childhood infection. It is to be noted, however, that a considerable period of time, possibly years in some instances, may elapse between reinfection and the appearance of tuberculosis in clinical form.

Bacteriological Diagnosis of Tuberculosis.[30]—Tubercle bacilli are discharged from the infected individual in sputum and urine and may be demonstrated in these materials, and in gastric washings, spinal fluid or infected tissues, depending on the location of the infection. Of specimens from these sources, those obtained by gastric lavage are of particular value in respiratory tuberculosis in children, who tend to swallow sputum, and are also useful in adult infections.[31] The presence of the bacilli may be shown either directly or after concentration by examination of stained smears, culture and guinea pig inoculation.

As a rule, some method of concentration is desirable, for not less than 100,000 bacilli per ml. must be present before there is a reasonable chance of finding them by microscopic examination. Because of the unusual resistance of the tubercle bacillus, the infected material can be treated with an equal volume of 3 per cent sodium hydroxide, or with antiformin (a proprietary preparation of hypochlorite) in final concentration of 15 per cent for fifteen to thirty minutes without killing them. The digested material is neutralized in the case of alkali treatment, centrifuged, and the sediment used for the preparation of smears and to inoculate guinea pigs and culture media. Chemical flocculation with alum or ferric chloride is desirable with specimens such as urine or spinal fluid, and may be applied to digested material to facilitate spinning out of the bacilli. A flotation method of concentration may be used which consists of dilution of the digested material with water and shaking with a hydrocarbon such as xylol or gasoline; the creamy layer separating out on standing is smeared and stained.

Smears are usually stained by the Ziehl-Neelsen method; fluorescent microscopy is of sufficiently recent development that it is not yet widely used though it may well become generally adopted. The demonstration of acid-fast bacilli allows a provisional diagnosis but does not indicate whether the bacilli were viable, or whether they are virulent tubercle bacilli. Their presence in urine specimens in particular must be interpreted with caution because of the frequent occurrence of the smegma bacillus which is also acid-fast.

Guinea pigs are inoculated in the groin or in the muscle of the thigh. If a reasonable number of tubercle bacilli have been inoculated, enlargement of the regional lymphatics may be noted in two or three weeks, the pig becomes emaciated by four to six weeks and usually dies not long after. With very small numbers of bacilli evidence of the infection may be delayed two or three weeks longer. If it is not obviously ill or does not die, the animal should be kept for eight weeks before sacrificing. At autopsy the regional lymph nodes will be found to be enlarged and filled with case-

[30] For the minimum laboratory standards set up by the American Trudeau Society, given in technical detail, see Amer. Rev. Tuberc., 1942, *45*:103.

[31] *Cf.* Davies and Doherty: Brit. Med. Jour., 1942, p. 212; Robinson and Dunn: Amer. Rev. Tuberc., 1943, *47*:413.

ous material. Necrotic areas in the spleen and liver are characteristic of the gross pathology in this animal; tubercles are seldom seen, and the lungs are only slightly affected and the kidneys almost never. Tubercle bacilli may be cultured from the lesions and found in acid-fast-stained smears. Some workers tuberculin-test the animals before inoculation and three to four weeks afterward, the development of a hypersensitivity indicating infection. If differentiation of the human and bovine varieties of the bacillus is desired, rabbits may be inoculated (see p. 586).

The culture of tubercle bacilli, carried out concurrently with animal inoculation, is becoming quite general and some workers feel that it is as sensitive as guinea pig inoculation.[32] As indicated earlier, a variety of media, containing glycerol and egg, may be used, the particular medium varying from one laboratory to another. A slant is inoculated heavily with the sediment from the digested specimen and the tube sealed with a cork or by dipping the end in hot paraffin to prevent evaporation. Characteristic colonies of tubercle bacilli appear after about three weeks' incubation.

Immunity.—The immune response of the animal body to the presence of the tubercle bacillus is indicated by the appearance of agglutinins, precipitins, opsonins and complement-fixing antibodies in the serum. This response is not marked, however, for these antibodies are present only to low titers. None of them, with the possible exception of the complement-fixing antibodies, is of diagnostic value.[33]

The most striking immune response is the development of a hypersensitivity to the bacillary cell substance. Within limits, this allergic response is protective against reinfection, as shown by Koch's[34] early experiments. He showed that the subcutaneous inoculation of the normal guinea pig with tubercle bacilli produces no immediate response but that in ten to fourteen days a nodule develops which breaks down to a persistent tuberculous ulcer and the regional lymph glands become swollen and caseous. In the tuberculous animal, however, an indurated area appears within a day or two, and there is slight necrosis with the formation of a shallow ulcer which heals promptly without the development of gross tubercle tissue or the invasion of the adjacent lymphatics by the bacilli. This resistance to reinfection is relative, of course, and is not shown unless the primary infection is of some weeks' standing or if large numbers of bacilli are injected. Animals may be sensitized, not only by infection with virulent bacilli, but also by the inoculation of attenuated or killed bacilli. Sensitization with preparations of the bacillary cell substance is difficult, however, and very large doses must be administered. The hypersensitivity is not passively transferable, and thereby differs from anaphylaxis.

Tuberculin.[35]—The sensitized animal will react to the soluble cell substance of the tubercle bacillus, preparations of which have been called *tuberculin*. Tuberculin is usually prepared from the human type of tubercle bacillus, though bovine tuberculin is practically as active as human tuber-

[32] See, for example, Petran and Perry: Jour. Lab. Clin. Med., 1939, *24*:539.
[33] *Cf.* Klopstock: Brit. Jour. Tuberc., 1941, *35*:146.
[34] Koch: Deut. med. Wchnschr., 1891, *17*:101.
[35] *Cf.* the discussion by Parish: Tubercle, 1938, *19*:337.

culin in infections with the human bacillus; avian tuberculin, although considerably less active, will also produce a reaction. A variety of tuberculins have been prepared of which only a few need be noted here. The first of these was made by Koch,[36] and consisted of the filtrate of a glycerol-broth culture of the bacilli concentrated by evaporation on a water bath to about one-tenth its original volume (the activity is heat-stable). This material is "original" or "old" tuberculin (TO or OT). A "new" tuberculin (TR—tuberculin residuum) was prepared by Koch in 1897[37] by macerating living virulent bacilli and extracting the mass with water, and then making an emulsion of the residuum. He later[38] advocated the use of an emulsion (BE—bacillary emulsion or *Bazillenemulsion*) of the entire substance of young virulent bacilli in 20 per cent glycerol, actually a vaccine. Denys[39] introduced the use of the unaltered filtrate from broth cultures (BF—broth filtrate). None of these later innovations, however, proved to be superior to old tuberculin and the original preparation, or slight modifications of it, has been widely used.

The active principle of tuberculin is protein in nature, and the cultivation of the tubercle bacillus in glycerol-asparagin-citrate synthetic solutions by Long and Seibert[40] has made possible the study of the active principle in purified preparations. The activity is associated with a number of protein fractions, one of which Seibert prepared in crystalline form. A more satisfactory preparation of low molecular weight, *ca.* 2000, has been isolated by Seibert by precipitation with trichloracetic acid. Originally designated SOTT (synthetic medium old tuberculin trichloracetic acid precipitated), it is now known as PPD (purified protein derivative).[41]

The comparative merits of OT and PPD have been the subject of a series of investigations in recent years. OT is relatively unstable in dilutions, while PPD, a dry powder, is "dry-diluted" with lactose and is, of course, indefinitely stable in this form. Different lots of OT vary somewhat in their activity; the activity of PPD preparations is relatively constant. It appears that PPD is quite as satisfactory as OT in actual use and, because of its stability and constant activity, is regarded by many as superior to OT.

The Tuberculin Reaction.[42]—Three types of reaction may be elicited in the sensitized, *i.e.*, infected, animal by the injection of tuberculin. In addition to a local inflammatory reaction at the site of inoculation, there is a focal reaction manifested as an acute congestion around tuberculous foci which, if marked, may aggravate the pathologic process, and a constitutional reaction in which the temperature rises to a peak of 102° to 104° F. and subsides in twelve to eighteen hours. In man the constitutional reaction also includes malaise, pain in the limbs and, perhaps, vomiting, dyspnea

[36] Koch: Deut. med. Wchnschr., 1890, *16*:1029; *ibid.*, 1891, *17*:101, 1189.
[37] *Ibid.*, 1897, *23*:209.
[38] *Ibid.*, 1901, *27*:829.
[39] Denys: Bull. méd. Paris, 1906, *20*:772.
[40] Long and Seibert: Amer. Rev. Tuberc., 1926, *13*:393.
[41] See the reviews by Seibert: Amer. Rev. Tuberc., 1941, *44*:1; Bact. Rev., 1941, *5*:69.
[42] See the general comprehensive discussion by Long: Amer. Rev. Tuberc., 1939, *40*:607.

and other symptoms. These reactions do not appear in normal animals. The utility of tuberculin is, then, two-fold; it may be used for diagnostic purposes and it has therapeutic value, though the latter is strictly limited.

The diagnostic tuberculin test in man is generally a skin test. Koch's original method consisted of subcutaneous injection of tuberculin. The cutaneous reaction of von Pirquet[43] involves the rubbing of tuberculin on to the scarified skin. In the Mantoux[44] test, the one most commonly used today, graded doses of tuberculin are injected intradermally, usually starting with 0.01 mg. of OT and going as high as 1.0 mg. or even 10 mg. on rare occasions. (0.1 cc. of a 1:100 dilution of OT is supposed to contain 1 mg.; the standardization of new batches is biological and carried out in guinea pigs infected with virulent tubercle bacilli.) Smaller amounts of PPD are used, since it is in dry, pure form; usually 0.00005 to 0.005 mg. A "patch test" has been introduced by Vollmer[45] in which squares (0.8 cm.) of thin filter paper, impregnated with tuberculin about four times as strong as the original old tuberculin and dried, are taped on the cleansed skin over the sternum or upper edge of the trapezius. The patch test appears to be somewhat less sensitive than the intracutaneous test of Mantoux. Since these are all skin tests only the local inflammatory reaction is observed in infected persons.

The diagnostic value of the tuberculin test in man is limited to young children, in whom it may be regarded as indicative of tuberculous infection. Once reactive to tuberculin, an individual generally remains reactive throughout life, although there are occasional reversions, and a positive tuberculin test has little significance in the adult. It may be noted that some work has indicated that the correspondence of a positive tuberculin reaction and either healed or clinically significant tuberculous infection may not be as close as is generally believed.[46]

The tuberculin test in cattle has great diagnostic importance, however, and has been widely used in the United States and to a lesser extent elsewhere. Three types of test may be used in cattle: the intradermal test; the ophthalmic reaction of Calmette, in which tuberculin is dropped into the conjunctiva and the reactive animal responds with the development of a diffuse congestion and edema in six to eight hours which fades away in twenty-four to thirty-six hours; and the constitutional reaction as indicated by a rise in temperature following the injection of tuberculin. The intradermal inoculation into the skin of the caudal fold is generally practiced in this country.

The therapeutic value of tuberculin may be directly observed in lupus or tuberculous infection of the skin. As indicated above, there is a reaction about the foci of infection manifested as an acute congestion and sloughing off of tissue. When it was first introduced tuberculin was regarded by

[43] von Pirquet: Ber. klin. Wchnschr., 1907, *48*:644, 699.

[44] Mantoux: Pr. méd., 1910, *18*:10.

[45] *Cf.* Volmer and Goldberger: Amer. Jour. Dis. Children, 1937, *54*:1019; *ibid.*, 1938, *56*:584; *ibid.*, 1939, *57*:1272; *ibid.*, 1939, *58*:527.

[46] Lumsden, Dearing and Brown: Amer. Jour. Pub. Health, 1939, *29*:25; Dahlstrom: Amer. Rev. Tuberc., 1940, *42*:471.

many as a highly effective specific therapeutic agent for tuberculosis. Its use is, however, exceedingly dangerous, and, with the exception of lupus, the response of tuberculous infection to the injection of tuberculin has been disappointing. The therapeutic use of tuberculin has diminished greatly in recent years. It may be noted that the minute quantities used in the tuberculin test do not affect tuberculous lesions.

The Mechanism of Immunity.—Infection with the tubercle bacillus confers a definite protection against reinfection, an "immunity to superinfection" resembling that observed in syphilis. The factors involved are as yet obscure. The antibodies produced are apparently not significant and antisera have no protective or curative properties. As indicated above, the development of hypersensitivity is the most obvious response to infection, and there is no doubt that hypersensitivity plays a part in acquired resistance as indicated in Koch's early experiments. Its relative importance is, however, not at all clear, and the mechanism of what seems to be a low-grade immunity of short duration remains at the moment largely a matter of speculation.

Active Immunization.—The possibility of active immunization to tuberculosis has been of great interest since the discovery of the tubercle bacillus. In general, two types of vaccines have been employed, suspensions of living attenuated bacilli and suspensions of killed bacilli.

The attenuated strain of the bovine tubercle bacillus, B.C.G., is regarded by some as an effective immunizing agent. Very young children are inoculated, preferably within a few days of birth, with suspensions of living bacilli. Such inoculation has been carried out on a wide scale in France and, to a very limited extent in this country, by Park and his associates in New York City. Since the incidence of tuberculosis is not great, an adequate test of the efficacy of such vaccination demands repeated observations on thousands of persons, both inoculated and control groups, over a period of years. In spite of the obvious practical difficulties, the French workers[23] claim that the incidence of tuberculosis in inoculated persons is significantly decreased. The statistical evidence on which this conclusion is based has been found, however, to be open to considerable criticism and is regarded by many as of little value. In general, it may be said that as yet no sound evidence involving adequate and comparable control groups has been presented, and the value of B.C.G. as an immunizing agent remains doubtful.

Perhaps to be regarded in the category of attenuated living bacilli is the "turtle bacillus" vaccine of Friedmann.[47] This microorganism, *Mycobacterium chelonei*, is of the "cold-blooded" variety and was isolated from a turtle. It is quite ineffective as an immunizing agent and is of only historical interest.

Suspensions of killed tubercle bacilli have been used in the immunization of cattle and experimental animals. The recently developed Spahlinger vaccine[48] is of this type. Although the resistance of guinea pigs and cattle appears to be raised by inoculation, the "immunity" so produced is of a

[47] *Cf.* Friedmann and Aronson: Jour. Inf. Dis., 1929, *44*:222.
[48] Spahlinger: Lancet, 1932, *i*:309.

very low order.[49] The inoculation of human beings with suspensions of killed bacilli has not been attempted on a large scale.

Pathogenicity for Lower Animals.—Tuberculosis of lower animals living under natural conditions is probably very rare. Animals in captivity, however, may contract the infection with some facility; in animals kept in zoos and in monkeys in the laboratory for experimental purposes tuberculosis is not uncommon. Domestic animals may be similarly affected. A wide variety of animals may, of course, be infected experimentally with one or another of the types of tubercle bacilli.

Domestic Animals.—The most commonly infected domestic animals are cattle, pigs and chickens. Cattle are infected with the bovine type of tubercle bacillus almost exclusively; they are not completely resistant to the human type as Koch originally thought, but infection is accomplished with some difficulty. The proportion of cattle infected increases with advancing age and, in the absence of control measures, may reach 70 to 90 and possibly 100 per cent in animals kept in stalls. The natural infection is generally of a chronic, slowly progressive nature. The lymphatics are most frequently involved and may be the only tissues to show lesions. The lungs are also commonly affected. Lesions on the pleura have a peculiar characteristic appearance, the so-called "perlsucht" disease. The liver, spleen and kidneys are less frequently involved, but infection of the mammary glands is not uncommon. It may be noted that tubercle bacilli may be excreted in the milk in the absence of detectable lesions of the mammae. Congenital tuberculosis in cattle occurs with some frequency.

Fig. 139.—Avian tubercle bacillus. Acid-fast stain of a smear from a pure culture. × 1050.

Tuberculosis in chickens is very common and is exclusively an infection with the avian variety of the tubercle bacillus. With the exception of parrots and certain birds of prey, birds are highly resistant to infection with the human and bovine varieties, and it is probable that natural infection

[49] *Cf. Report on the Spahlinger Experiments in Northern Ireland.* H. M. Stationery Office. London. 1935.

with these types rarely if ever occurs. Tuberculosis in chickens is usually a chronic process and is characterized by the formation of nodules in the abdominal viscera. The lungs are less frequently affected.

Pigs suffer from natural infection with both bovine and avian tubercle bacilli derived from infected cattle and poultry; they are also susceptible to infection with the human variety. In young pigs infection with bovine bacilli is generalized and acute with lesions in the lymphoid tissue, abdominal viscera and lungs. Infection with the human bacillus is generally localized, but the avian type may produce a generalized infection.

Other domestic animals suffer from tuberculosis to a considerably lesser extent. Horses, dogs and cats are occasionally infected, and the disease is rare in sheep and goats.

Experimental Animals.—Experimental animals vary in their susceptibility to the varieties of the tubercle bacillus and in the type of infection

CHARACTERISTICS OF THE VARIETIES OF TUBERCLE BACILLI

Variety	Physiology				Pathogenicity		
	Opt. temp.	Rate of growth	Pig-ment	Stimula-tion by glycerol	Guinea pig	Rabbit	Chicken
Human	37°C.	eugonic	+	+	++++	±	−
Bovine	37° C.	dysgonic	−	−	++++	++++	−
Avian	40–42° C.	rapid	+	+	+	++	++++

produced. Of these animals only two need be considered here, the guinea pig and the rabbit. The guinea pig is highly susceptible to both bovine and human bacilli, and death follows the subcutaneous injection, even of small doses, in six to fifteen weeks. The lymphatic glands, spleen and liver are most affected, the lungs only slightly and the kidneys never. The necrotic areas in the spleen and liver are the most striking feature of the gross pathology and are peculiar to the guinea pig. True tubercles are seldom seen except in the very early stages of the disease.

Rabbits are highly susceptible to infection with the bovine bacillus and quite resistant to the human variety, even more so than to the avian bacillus. Injection of bovine bacilli produces a generalized infection that terminates fatally in two to three months. On autopsy tubercles may be found in the spleen and liver, but the lesions are most marked in the lungs and kidneys and may even be confined to them. Very large doses of human bacilli (10 to 50 mg. intraperitoneally) may produce a progressive infection but never the acute fatal miliary disease. The mammalian tubercle bacilli, indistinguishable by cultural or serological methods, may, then, be sharply differentiated by their pathogenicity for these two experimental animals.

Neither rabbit nor guinea pig is particularly susceptible to infection with the avian bacillus though avian tuberculosis may be produced in the

rabbit. In the guinea pig death may be produced by large intraperitoneal doses, but on autopsy macroscopic tubercles are not visible; cultures and smears of the liver and spleen, however, show the presence of the bacilli. This form of tuberculosis, the proliferation of the bacilli without macroscopic tubercle formation, is known as the *Yersin type* of tuberculosis.

Epidemiology.—In man tuberculosis is largely an air-borne infection. Dissemination by milk is of relatively minor importance, particularly in the United States. The disease is one of civilization in that its transmission is facilitated by close contact. The frequency of positive tuberculin reactions rises rapidly from zero in the newborn to perhaps 90 per cent or over at puberty, and, as indicated earlier, almost all adults have been infected

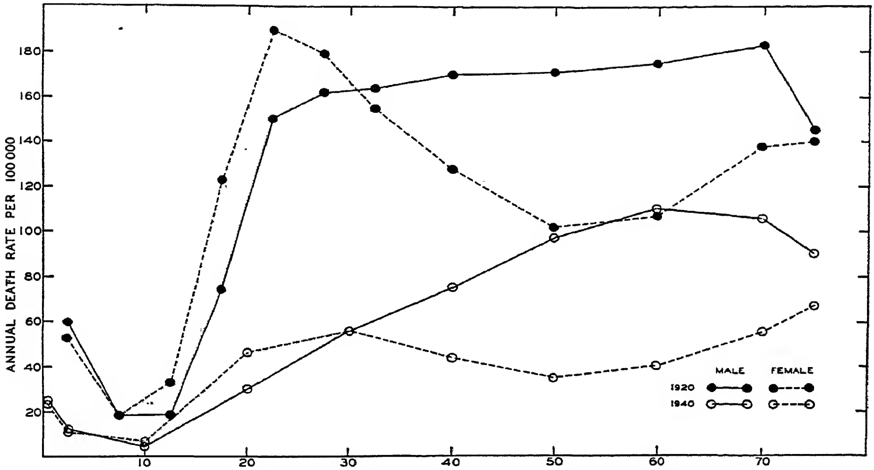


Fig. 140.—The age and sex distribution of tuberculosis mortality (all forms) in 1920 and 1940. Note the differential incidence and decline over the twenty-year period. If differentiation between white and colored rates is made for 1940, the picture is almost the same, *i.e.*, the colored rates are very similar to the 1920 rates for both white and colored. Data from the reports of the Census Bureau.

at one time or another. The infection is, then, widely disseminated through the human population. In most instances, however, clinical tuberculosis has not occurred and the lesions have healed. The proportion of persons with active clinical tuberculosis is not known; in some investigations the ratio of cases to deaths has been found to be as high as 10:1 or 12:1, but the ratio of reported cases to deaths is not much more than half of this. The prevalence of tuberculosis, then, defies precise definition.

There appear to be racial differences in susceptibility to tuberculosis. The death rate of the Negroes in this country is considerably higher than that of the white population, though the incidence of clinical tuberculosis in the two races is not greatly different. Whether this higher death rate is a consequence of environmental conditions or whether it is in part attribu-

table to racial differences in susceptibility has been a point of considerable interest. It is probable that both factors are involved and that there is a real racial difference in susceptibility. In this connection the experience in the United States Army is of particular interest. Roth[50] has found that in the years 1922–36 the average white morbidity rate was 2.10 per 1000 and the Negro rate 2.56, a ratio of 4:5; the white death rate was 0.24 and that of the Negroes 0.99, a ratio of 1:4; and the case-death ratios were 8.75 for the whites and 2.61 for the Negroes. Under the controlled conditions prevailing, *i.e.*, preliminary physical examination, age and sex selection, the same housing conditions and identical diagnostic and therapeutic facilities, it would appear to be definitely established that though the incidence of clinical tuberculosis is no greater in the Negro, the disease is much more frequently fatal and the indicated greater susceptibility is a consequence of racial rather than environmental factors. There is some evidence that less well-defined races of man differ in their resistance to tuberculosis; Jews and Italians appear to be more resistant than the Irish (p. 188).

The age and sex distribution of mortality from tuberculosis must be considered in relation to the decline in the disease rather than as isolated phenomena. That tuberculosis has been decreasing at a steady and relatively rapid rate since 1850 or thereabouts is indicated by the decline in the death rate from this disease. As shown in Fig. 141, the death rate from all forms of tuberculosis in the registration area of the United States has declined from 180.5 per 100,000 in 1900 to 43.0 in 1942, and a decline of this general order is apparent elsewhere. As in the case of some other diseases, the decline set in before the discovery of the bacterial etiology of infectious disease and the development of preventive measures, and hence is by no means entirely attributable to the practice of preventive medicine.

This decline has not been relatively the same in either the various age groups or in the two sexes. The death rate is highest in the very low age groups, that of one to two years, and falls rapidly in the five to nine group and then rises to a peak in early adult life, twenty to twenty-four, and declines with, in recent years, a small secondary peak between forty-five and fifty-four years (late adult tuberculosis). This secondary peak is a relatively recent development and is regarded by Frost[51] as a residual of higher rates in earlier life rather than a postponement of maximum risk. The decline in tuberculosis in the present century has been relatively greatest in the very young, an undoubted consequence of preventive measures.

The sex distribution of the death rates from tuberculosis in the various age groups is a curious phenomenon which has not been explained. The death rate for males of all ages is somewhat higher than that for females. In young adults, *i.e.*, the fifteen to twenty-nine age group, the female death rate is considerably higher than the male death rate. In the higher age groups the male death rate becomes proportionately greater and exceeds the female rate for the rest of life. In the present century the female

[50] Roth: Amer. Rev. Tuberc., 1938, *38*:197.
[51] Frost: Amer. Jour. Hyg., Sec. A, 1939, *30*:91.

death rate in the higher age groups has declined somewhat more rapidly than the male death rate. The present status of the relation between the sex-specific death rates may be illustrated by the ratio of male to female deaths in the United States registration area in 1931–34 as given by Dauer.[52] For all ages the ratio of male to female deaths was 1.24, at ages under ten the ratio was 1.22, at ten to thirty years 0.69, at thirty to fifty-five years 1.64, and at fifty-five and over 1.57. (See Fig. 140.)

Tuberculosis mortality has fallen at a more accelerated rate than deaths from all causes; in 1900 more than 11 per cent of all deaths were due to tuberculosis, in 1940 the percentage had fallen to 4.3. At the beginning of the century tuberculosis was first in numerical importance as a cause of death and it was seventh in 1940. Although the reported cases and deaths

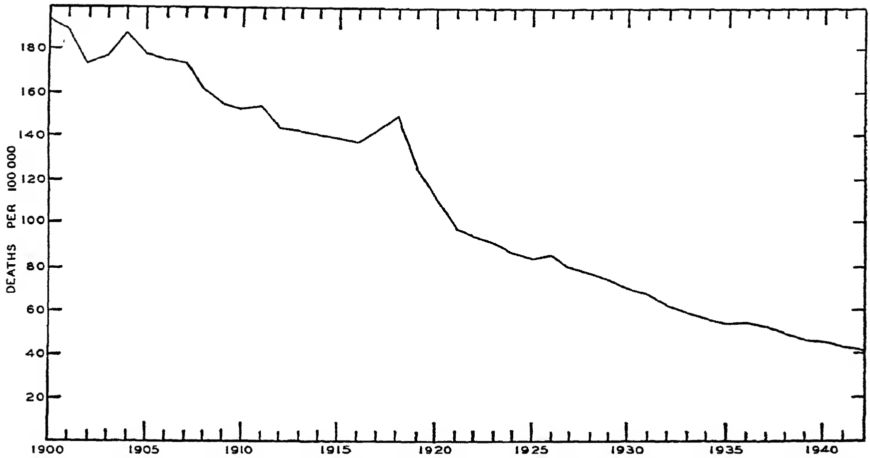


Fig. 141.—The prevalence of tuberculosis, all forms, in the Registration Area since 1900 as indicated by the uncorrected death rates. From data compiled by the Bureau of the Census.

have declined markedly, there has not been any appreciable alteration in the proportion of cases to deaths, *i.e.*, the case-fatality rate.[53]

It will be clear that the observed decline in tuberculosis is a complex matter for which there is no simple explanation. Although unknown factors are undoubtedly involved, it appears highly probable that the environmental improvements implied in the more decent living of the last few decades have contributed in no small part to the decrease in tuberculosis. The early detection and isolation of cases practiced in recent years has very likely had its effect also. Frost[54] has pointed out that the observed decline is indicative of the inability of the disease to reproduce itself; a surplus of chances to establish infection are, of course, essential and the

[52] Dauer: Amer. Rev. Tuberc., 1938, *37*:435.
[53] *Cf.* Drolet: Amer. Rev. Tuberc., 1938, *37*:125.
[54] Frost: Amer. Rev. Tuberc., 1935, *32*:644.

required level of transmission may be very high and necessitate not only universal but frequently repeated exposure to relatively large doses of bacilli. If the requisite level is not maintained the disease must necessarily die out. In certain other diseases the causative agent may be disseminated by healthy persons; in tuberculosis, however, it is only the "open" clinical case that discharges bacilli into the adjacent environment. The present practice of isolation of active cases would appear, then, to be a sound one, and it is perhaps not unreasonable to hope for the eventual eradication of this disease.

THE BACILLUS OF LEPROSY (MYCOBACTERIUM LEPRAE)[55]

Like tuberculosis, leprosy is an old disease of man. Perhaps more prevalent in ancient times, it is now most common in Central Africa, India, Japan and other Asiatic countries, and it was estimated that there were about 3,000,000 lepers in the world in 1924. The disease is prevalent in South America, with endemic centers in Brazil, Colombia and Argentina, and an over-all incidence of more than 1 per 1000 population. Practically all the Caribbean islands are infected to about the same extent. It is relatively uncommon in Europe and sporadic cases occur in Latvia, Estonia, southern and eastern Russia, and on the Mediterranean coast.[56]

Leprosy has been introduced into the United States with varying consequences. In Louisiana, Florida and Texas the imported cases have established foci in which the disease has a tendency to perpetuate itself, while in California and the central northwestern States it tends to die out. Elsewhere in the country transmission is so rare as to be negligible.[57] It is estimated that there are 500 to 1000 lepers in this country, many of whom are segregated in the National Leprosarium in Carville, Louisiana, the remainder living for the most part in California and New York.

Bacilli were found by Hansen[58] in 1872 in the round epithelioid cells generally known as lepra cells, and the observation was one of the first of pathogenic bacteria.

Morphology and Staining.—Morphologically the leprosy bacilli closely resemble the tubercle bacilli. They are long (6 μ), slender rods, usually straight, but sometimes slightly curved. They are non-motile and do not produce spores. They generally occur within the cells but are sometimes found free in the lymph spaces. Their arrangement within the cells is characteristic, several bacilli being usually grouped together in bundles like packets of cigarettes.

The staining reaction of these microorganisms is much like that of the tubercle bacilli. They stain somewhat more readily than the latter, and also decolorize more quickly with acids, but the difference is not great enough for differentiation. The presence of large numbers of bacilli within the cells, together with the clinical features of the disease, makes it pos-

[55] For a detailed consideration see Rogers and Muir: *Leprosy*. Williams & Wilkins Company, Baltimore, 1940.

[56] *Cf.* Saunders: Proc. Sixth Pacific Sci. Congr., 1942, *5*:957.

[57] McCoy: Pub. Health Repts., 1942, *57*:51.

[58] Hansen: Norsk Mag. f. Laegevidensk., 1872, *2*:1.

sible to distinguish leprosy bacilli from tubercle bacilli without difficulty. Because of their acid-fast staining characteristic and their morphological resemblance to the tubercle bacilli and similar bacteria, these bacilli are included with the mycobacteria and designated *Mycobacterium leprae.*

Cultivation.—Numerous unsuccessful attempts to cultivate Hansen's bacillus on artificial media were made for years by bacteriologists all over the world. A few investigators have reported positive results. In most instances acid-fast bacilli have been cultivated, but in others a variety of microorganisms, including diphtheroids, actinomycetes and anaerobic bacilli have been found. It has been suggested by some workers that the acid-fast bacilli present in leprous lesions represent but a single stage in a developmental cycle and outside the body other forms appear. Kedrowski[59] cultivated a highly pleomorphic microorganism with branched, acid-fast and non-acid-fast stages. Clegg[60] also obtained apparent growth and con-

Fig. 142.—The leprosy bacillus. Acid-fast stained smear from a skin lesion. Note the characteristic tendency to parallel arrangement of the bacilli in packets. × 1800.

tinued multiplication in subculture on an artificial medium of an acid-fast bacillus. His results were confirmed by others, in particular Duval,[61] who reported cultivating a bacterium regarded as *Myco. leprae* directly from human tissue on artificial media. Acid-fast bacilli, some of them chromogenic, have been cultivated by other workers, and there are at present a number of cultures in various laboratories labeled "*Myco. leprae.*"

It seems highly probable that not any of these bacteria are leprosy bacilli in that they are etiologically related to the disease in man.[62] They are very likely best grouped with the saprophytic acid-fast forms such as the smegma and timothy bacilli.

[59] Kedrowski: Ztschr. f. Hyg., 1901, *37*:52.
[60] Clegg: Philippine Jour. Sci., 1909, *4*:403.
[61] Duval: Jour. Exp. Med., 1910, *12*:649.
[62] Four strains of "leprosy bacilli" isolated in recent years were intensively studied at the National Institute of Health, but there was no evidence of a causal relation to the disease. *Cf.* Nat. Inst. Health Bull. No. 173, 1940.

The cultivation of the bacilli of leprosy has been intensively investigated in recent years by Soule and McKinley, who, in 1932,[63] first isolated in culture a very slow-growing, non-chromogenic acid-fast bacillus which closely resembled the bacilli observed in leprous nodules. On media suitable for the growth of tubercle and other acid-fast bacilli incubated in an atmosphere of 40 per cent oxygen and 10 per cent carbon dioxide for six weeks, tiny, discrete colonies appeared. Although a number of isolations failed to grow on subculture, 2 strains have been maintained through 40 transfers over a period of six years.[64] These cultures were obtained from leprous material in Puerto Rico. Two years later Soule[65] reported that of 42 specimens obtained in the Philippines, 25 yielded cultures of the same or a very similar bacterium, and of these 2 strains were maintained on repeated subculture. The intradermal inoculation of *rhesus* and *Cebus* monkeys resulted in the production of temporary granulomatous lesions which could not be transmitted in series. Whether these bacilli are, in fact, leprosy bacilli is not clear; proof demands the reproduction of the disease, but since inoculation of experimental animals with relatively large amounts of macerated leprous nodules has not produced the disease, the negative results with cultures are, perhaps, not significant.[66] More recently Loving[67] has reported the cultivation of the bacilli in a thiamin-enriched medium.

Pathogenicity.—Although any organ or tissue in man may be attacked with varying results, two distinct types of leprosy are usually recognized—the nodular and the anesthetic. The former, which is the more acute, is

RELATIVE PROPORTION OF TYPES OF LEPROSY*

Region	Neural Type	Lepromatous Type
Africa	90.5%	9.5%
Philippines	50.0%	50.0%
Mexico	40.0%	60.0%
Java	29.0%	71.0%

* Data from Lowe: Proc. Sixth Pacific Sci. Congr., 1942, 5:921.

characterized by the development of masses of granulation tissue, the so-called "leproma," which may appear superficially in different parts of the body, and by their growth and coalescence produce distortion and mutilation. The anesthetic type, or nerve leprosy, progresses more slowly, the average duration of the cases being nearly twice as long (eighteen years) as cases of the nodular type, some being known to extend over thirty-five or forty years. Atrophy of the muscles and other trophic disturbances accompany the nerve lesions.

[63] Soule and McKinley: Amer. Jour. Trop. Med., 1932, *12*:1, 441; McKinley and Soule; Jour. Amer. Med. Assn., 1932, *98*:361.
[64] *Cf.* Soule and McKinley: Amer. Assn. Advncmt. Sci., Symposium Ser., 1938, *1*:87.
[65] Soule: Proc. Soc. Exp. Biol. Med., 1934, *31*:1197.
[66] *Cf.* the review by McKinley: Int. Jour. Lepr., 1939, *7*:1,217.
[67] Loving: Amer. Jour. Trop. Med., 1943, *23*:593.

In both forms of leprosy Hansen's bacillus is found in all cases, in enormous numbers as a rule, in the lesions of nodular leprosy and less abundantly in the anesthetic type. Very few bacilli are observed outside the body cells, and they are found in the cytoplasm and do not invade the nucleus. Almost any part of the body may be the site of leprous growth; the kidneys are usually invaded, the liver and spleen always. The bacilli have been seen in the central nervous system and are occasionally found in the blood, generally in the leucocytes but sometimes free.

Leprosy has never been produced in experimental animals by the inoculation of leprous material from man; even the implantation of nodules containing enormous numbers of bacilli has been without effect.

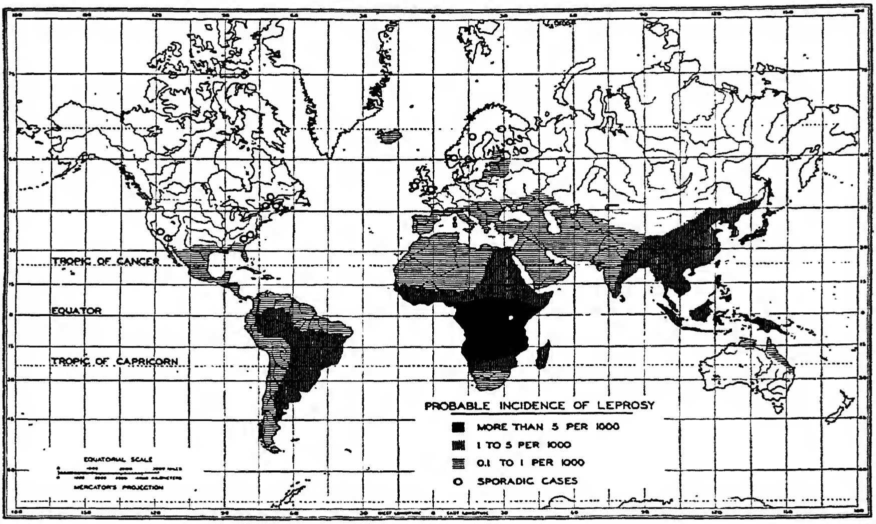


Fig. 143.—The probable prevalence of leprosy in the world as indicated by present data. Based on Goode *Base Map* No. 201 M. By permission of the University of Chicago Press. (After Saunders.)

Transmission.—Leprosy is probably transmitted by contact, though the conditions that make transmission possible are entirely unknown. There are numerous instances in which healthy persons, such as asylum attendants, have been more or less in contact with lepers for long periods without contracting the disease. In other cases, however, leprosy has been contracted by those in close and long-continued contact with diseased individuals. There is also indirect evidence of transmission. Manson[68] cites the case of an Irishman who acquired leprosy in the West Indies. On his return to Ireland his bed was shared by his brother who, moreover, sometimes wore the leper's clothes. The brother, who had never been in any foreign country, became in time an undoubted leper. In this instance communication from one person to another was practically demonstrated.

[68] Manson: *Tropical Medicine*. London. 1900, p. 448.

Currie[69] found that a large percentage of cases studied in Hawaii gave a history of exposure, and that usually such exposure was of an intimate character. Saunders[70] has pointed out that leprosy occurs for the most part in populations of low social order living under unsanitary conditions of poverty, crowding, uncleanliness and sickness. Even so a relatively small proportion of those exposed develop the disease.

One way in which the bacillus may leave the body is in the nasal mucus, and large numbers of the bacilli are found in the secretions of the nose in many cases. Bacilli may sometimes be discharged from the mouth or nose in small particles of mucus. In the opinion of many writers, the mucous membrane of the nasopharynx is the point at which the bacteria are introduced into the body, as well as the chief source from which infection is spread.

Evidence of the direct inoculability of leprosy from man to man is quite inadequate. Many attempts to infect healthy persons have been made and have failed, and one often-cited instance of successful inoculation is by no means unimpeachable. In the case of the criminal Keanu in the Hawaiian Islands, reported by Arning,[71] implantation of material from a leprosy nodule was followed by the development of true leprosy, which terminated fatally six years after inoculation. The experiment, however, did not exclude the important source of error involved in the facts that Keanu was a native of a country in which leprosy was common, that he had lived among lepers, and that members of his family were lepers. More recently Lazoudaky[72] inoculated himself intramuscularly with blood from two lepers and contracted the disease.

Much light is thrown on the contagious character of leprosy by the success that has attended the isolation and segregation of leprous patients. The Norwegian experience showed that a careful but not unduly rigorous system of separation was accompanied by a diminution of the number of cases from 2870 in 1856 to 577 in 1900. The circumstance that infection does not invariably follow chance contact or association should not, therefore, lead to neglect of the facts that leprosy is a bacterial disease; that up to the present under natural conditions the specific bacterium has not been found except in the human body; and that, so far as is definitely known, the leper himself is the only means by which leprosy spreads.

Immunity.—Man is clearly highly resistant to infection with the leprosy bacillus, and, after infection is established, the ensuing disease is essentially a benign process that may take many years to terminate fatally; in fact, many lepers die from other causes. Little is known regarding the specific immune response. Lepers do, however, develop a hypersensitivity to the cell substance of acid-fast bacilli such as the tubercle bacillus and the various saprophytic species. A curious fact is the appearance of a positive Wassermann reaction in lepers in the absence of syphilis. According to some, this is an artifact arising from infection with yaws, and it is

[69] Currie: Pub. Health Bull. No. 41, 1910.
[70] Saunders: Proc. Sixth Pacific Sci. Congr., 1942, *5*:957.
[71] Arning: Arch. f. path. Anat., 1893, *134*:319.
[72] Lazoudaky: Jour. Trop. Med. Hyg., 1937, *40*:77.

said that the Kahn test is negative in non-syphilitic lepers. Recent investigations,[73] however, have shown that both the Wassermann and the Kahn tests may be positive in lepers having no clinical symptoms of syphilis.

Lepromin.—The hypersensitivity developed by the leper may be demonstrated by the intradermal inoculation of material prepared from leprous nodules. Two reactions are observed, one early and occurring after three or four days, and the late reaction appearing three to four weeks after inoculation. The reaction is not specific in that a response is also elicited by preparations of other acid-fast bacilli. Dharmendra[74] has isolated a nucleoprotein from leprosy nodule material, however, which appears to have some specificity. In its present form, at least, the lepromin test has little or no diagnostic utility.

Rat Leprosy.—A native disease of wild rats, commonly known as rat leprosy, and characterized by enormous numbers of acid-fast bacilli present in the lesions, was described by Stefansky[75] in 1903, as occurring in wild rats in Odessa. The disease was observed in the same year by Dean,[76] who later[77] showed it to be transmissible. It has since been observed in wild rats all over the world.[78]

The acid-fast bacilli closely resemble the leprosy bacillus in size and shape and are found intracellularly but not as often in the packet arrangement of parallel bacilli. *Myco. leprae murium* has not been cultivated on artificial media. The disease may, however, be transmitted to white rats, mice and guinea pigs by inoculation with pieces of tissue.[79] The experimental infection is a relatively benign process. A local, circumscribed lesion develops following subcutaneous inoculation which becomes palpable in four to five weeks and eventually develops to a large tumorous mass ulcerating on the surface and persisting throughout the life of the rat. The earliest lesions in the other organs do not appear before four to six months, and the animal dies only after a year or more.

The relation of rat leprosy to human leprosy is not known. Rats are not susceptible to inoculation with human leprous material.

OTHER ACID-FAST BACILLI

Mycobacterium Paratuberculosis.[80]—A chronic enteritis of cattle usually terminating fatally is caused by an acid-fast bacillus closely resembling the avian variety of the tubercle bacillus. The disease is sometimes called *Johne's disease* and the bacillus *Johne's bacillus* after its discoverer.[81] The disease only remotely resembles tuberculous infection. The

[73] Badger, Patrick, Fite and Wolfe in Nat. Inst. Health Bull. No. 173, 1940; Faget and Ross: Ven. Dis. Information, 1944, *25*:133.

[74] Dharmendra: Leprosy in India, 1941, *13*:40, 77, 81; Indian Jour. Med. Res., 1943, *31*:125, 129.

[75] Stefansky: Centralbl. f. Bakt., 1903, *33*:481.

[76] Dean: Centralbl. f. Bakt., 1903, *34*:222.

[77] Dean: Jour. Hyg., 1905, *5*:99.

[78] See the review by Lowe: Int. Jour. Lepr., 1937, *5*:311, 463.

[79] For recent studies on experimental rat leprosy see Fite in Nat. Inst. Health Bull. No. 173, 1940.

[80] Hagan and Thomson: Tr. Nat. Tuberc. Assn., 1931, p. 232.

[81] Johne and Frothingham: Deut. Ztschr. f. Thiermed., 1895, *21*:438.

lesions in the intestinal wall are proliferative and the granulomatous tissue may contain epithelioid cells and occasionally giant cells, but there is no caseation.

The disease appears to be widespread in the United States.[82] Infected cattle become hypersensitive to the bacillary substance and filtrates of cultures produce a skin reaction analogous to the tuberculin reaction which is designated the "johnin reaction." No case of human infection with *Myco. paratuberculosis* has been recorded.

The Vole Bacillus.—An acid-fast bacillus responsible for an epizootic, chronic infection of the field vole, *Microtus agrestis*, resembling tuberculosis was discovered by Wells.[83] It closely resembles the tubercle bacillus culturally[84] though it forms no pigment and growth is not enhanced by glycerol. It is pathogenic for both guinea pigs and rabbits, considerably more so for the latter, and is not pathogenic for fowls.[85] Brooke[84] has suggested that it is a distinct type of mammalian tubercle bacillus and should be called *Mycobacterium tuberculosis* var. *muris*. This microorganism has been of particular interest because, though it produces only a localized and retrogressive infection when inoculated in small doses in guinea pigs and calves, tuberculin sensitivity is produced, and preliminary experiments on its use as a prophylactic have given suggestive results.[86]

The "Cold-Blooded" Mycobacteria.—Acid-fast bacilli have been found associated with pathologic processes in various cold-blooded animals. In some instances the processes superficially resemble tuberculous lesions. *Myco. piscium* was isolated from nodules and tumor-like formations in carp; *Myco. marinum* from "tuberculosis" of sea bass and certain other salt-water fish; *Myco. ranae* was found in the liver of a frog; *Myco. thamnopheos* is a parasite in garter snakes; and *Myco. chelonei*, the "turtle bacillus," has been referred to above.

The saprophytic acid-fast bacilli include the well-known timothy bacillus, *Myco. phlei*, found in soil, on grasses and elsewhere in nature; the "butter bacillus," *Myco. butyricum*; and *Myco. smegmatis*, which is, however, a parasite found in both male and female smegma. The smegma bacillus is often difficult to distinguish from the tubercle bacillus on morphological grounds, and confusion of the two may have considerable practical importance in the diagnosis of suspected cases of tuberculous infection of the urinary tract. It is, it may be noted, also found in the urine and may contaminate fecal specimens. The saprophytic bacilli all grow much more rapidly than the tubercle bacilli, and neither they nor the bacilli isolated from cold-blooded animals are pathogenic for guinea pigs and rabbits, or at best only feebly so. The interrelationships of these nonpathogenic acid-fast bacilli are considered at length by Gordon[87] and Gordon and Hagan.[88]

[82] Hastings, Beach and Mansfield: Wis. Agr. Exp. Station Res. Bull. No. 81, 1927.
[83] Wells: Lancet, 1937, *i*:1221.
[84] Brooke: Amer. Rev. Tuberc., 1941, *43*:806.
[85] Griffith: Jour. Hyg., 1942, *42*:527.
[86] Griffith and Dalling: Jour. Hyg., 1940, *40*:673.
[87] Gordon: Jour. Bact., 1937, *34*:617.
[88] Gordon and Hagan: Jour. Bact., 1938, *36*:39.

CHAPTER 32

MEDICAL MYCOLOGY: THE PATHOGENIC ACTINOMYCETES, MOLDS, YEASTS AND RELATED MICROORGANISMS[1]

It has been pointed out in preceding chapters that the tubercle bacilli and the diphtheria bacilli are more closely related to the fungi than are the "true bacteria." These forms may be regarded as connecting links between the bacteria and a number of the fungi proper, some of which are pathogenic for man and lower animals. The discovery of the causal relation of certain of the fungi to infectious disease preceded the pioneer work of Pasteur and Koch with the pathogenic bacteria by several years, for Schoenlein studied the fungus causing favus in 1839, and in the same year Lagenbeck described the yeast-like microorganism of thrush. In spite of its earlier beginnings, medical mycology was soon overshadowed by bacteriology and has never received as much attention though some of the fungus diseases are among the more common infections of man. This is perhaps attributable to the relatively benign nature of the common mycoses and the rarity of the more serious ones, and to the morphological basis of the differentiation of these structurally complex forms which, in a practical sense, sets them off sharply from the bacteria.

Even a brief consideration of the fungus diseases makes it clear that they fall into two well-defined groups, the superficial mycoses and the deep-seated mycoses.[2] The superficial mycoses are by far the most common, are caused for the most part by a relatively homogeneous group of fungi, the *dermatophytes*, and include the various forms of tinea or ringworm with infection of the hair and hair follicles, the superficial infections of the intertriginous or flat areas of the glabrous skin, and the onychomycoses or fungus infections of the toe and fingernails. In general, the lesions are mild, superficial and restricted; the infections are almost never fatal. The causative microorganisms are parasitic and the infection is

[1] For a general discussion of medical mycology see: Henrici: *Molds, Yeasts and Actinomycetes*. John Wiley & Sons. New York. 1930. A detailed consideration of the pathogenic fungi with little or no critical evaluation of their relative importance is given by Dodge: *Medical Mycology*. C. V. Mosby Company. St. Louis. 1935. An excellent, though largely clinical, discussion of the dermatophytes is presented by Lewis and Hopper: *Introduction to Medical Mycology*. Year Book Publishers. Chicago. 1939. A very brief discussion of the general subject is given by Swartz: *Elements of Medical Mycology*. Grune & Stratton. New York. 1943. Clinical and diagnostic aspects of the mycoses are discussed in detail in Conant *et al.*: *Manual of Clinical Mycology*. W. B. Saunders Company. Philadelphia. 1944. Recent literature is reviewed by Emmons: Bot. Rev., 1940, *6*:474.

[2] The general characteristics of the fungus diseases are discussed by Henrici: Jour. Bact., 1940, *39*:113.

transmitted from one host to another. The deep-seated mycoses, on the other hand, are relatively rare and are heterogeneous in etiology; they include actinomycosis, aspergillosis, sporotrichosis, blastomycosis, coccidioidomycosis and cryptococcus or torula infections. The causative microorganisms appear to be free-living parasites for the most part and are usually not transmitted from one individual to another, but the lesions they produce are severe, deep and spreading, and a fatal outcome is not uncommon.

THE FUNGUS INFECTIONS

Type	Causative Organism	Disease
Superficial infections	Dermatophytes: *Microsporum, Trichophyton, Achorion, Epidermophyton, Endodermophyton*	Ringworm of scalp and glabrous skin, suppurative folliculitis, onychomycosis
	Actinomyces minutissimus	Erythrasma
	Erysipelothrix rhusiopathiae	Erysipeloid in man
	Fonsecaea pedrosoi and its varieties	Chromoblastomycosis
	Candida albicans and related forms	Moniliasis of the mucous membranes and nails, sometimes generalized
Deep-seated infections	*Actinomyces bovis, A. asteroides, A. madurae*, etc.	Actinomycosis, including actinomycotic mycetoma
	Actinomyces muris-ratti	Rat-bite fever
	Aspergillus fumigatus, and others	Aspergillosis, otomycosis and pulmonary mycosis
	Sporotrichum schencki	Sporotrichosis
	Debaryomyces neoformans	European blastomycosis, torula meningitis
	Blastomyces dermatitidis	American blastomycosis
	Coccidioides immitis	Coccidioidomycosis
	Histoplasma capsulatum	Histoplasmosis

The criterion of pathogenicity is one of the poorest that can be used in the differentiation of microorganisms, not only because it is variable and difficult to determine, but because by its use parasitic microorganisms are grouped together that are, in fact, much more closely related to certain free-living forms than they are to one another. The superficial nature of pathogenicity as a differential characteristic is nowhere better illustrated than among the fungi, for the pathogenic forms which constitute the subject matter of medical mycology form a heterogeneous group and include some of the actinomycetes, certain molds and mold-like fungi, and a number of yeasts and yeast-like organisms. The pathogenic varieties of these fungi are an almost infinitesimally small portion of existing fungi,

the vast majority being saprophytic forms found in the soil and elsewhere playing a role of primary importance in the cyclical transformations of organic matter, especially in the decomposition of hemicelluloses and lignins. A number are of great industrial importance, as in cheese manufacture, saccharification of starches, etc. From a general biological point of view, then, the pathogenicity of certain fungi is of very minor significance; from that of the parasitized host, man, it is of considerably greater interest.

The fungi are structurally complex, showing a variety of reproductive structures associated with sexual and asexual processes, in addition to vegetative non-reproductive elements. Their differentiation into genera, species and varieties is made in large part on a morphological basis, especially the morphology of the reproductive structures, and, in contrast to the bacteria, their physiological and immunological characteristics are usually of minor or no importance for purposes of differentiation or identification. The biochemistry of the fungi, and the molds in particular, has been extensively investigated, beginning with the work of Wehmer and more recently by Raistrick and his colleagues[3] in connection with the elucidation of decompositions occurring in nature and their application to industrial processes. Present knowledge of the respiratory mechanism of the cell (p. 82) and of alcoholic fermentation (p. 102) derives in very large part from studies of yeasts. The immunological properties of the fungi have been little studied aside from noting the immunological relation of certain of the actinomycetes to the tubercle bacilli, and the allergic phenomena associated with infection with certain of the dermatophytes and yeast-like fungi.

Though a great many species of fungi have been described as pathogenic for man and animals, not all are of equal importance. Some, especially among the dermatophytes, are not legitimate species different from those already known. Furthermore, in many cases the fungus described probably had no causal relation to the pathologic process from which it was isolated and in others only one or two cases of infection have ever been observed. It is the accumulation of large numbers of species of fungi and the minutiae of their morphological differentiation that give medical mycology its complexity. Here we shall be concerned only with the more important fungi known to be causally related to human disease; the remainder, though large in number, are associated with only a small fraction of one per cent of fungus disease of man.

I. THE ACTINOMYCETES

The actinomycetes occupy an uncertain position with respect to the bacteria on the one hand and the fungi on the other and are regarded by many as transition forms. The order *Actinomycetales* is made up of two families, the *Mycobacteriaceae* which includes the mycobacteria and the corynebacteria, and the *Actinomycetaceae* which includes *Actinomyces*,

[3] *Cf.* Raistrick *et al.: Studies in the Biochemistry of Microorganisms*, Phil. Trans. Roy. Soc., 1931, Ser. B, *220*:1–367. Later papers will be found for the most part in the Biochemical Journal.

Erysipelothrix and *Leptotrichia*; the last is made up entirely of saprophytic forms and does not concern us here.

The actinomycetes grow in the form of five straight or wavy nonseptate filaments or hyphae 0.5 to 0.8 μ in diameter which show both lateral and dichotomous branching, and which may grow out from the medium to form an aerial mycelium. On semisolid media the filaments occur in tangled masses while in liquid media there is a tendency to centers or clumps of growth. Small oval to rod-shaped spores 1 to 2 μ long are formed by aggregation of the protoplasm in some of the hyphae, spore formation progressing from the tip. The maturation of the spore-bearing hyphae is often associated with the formation of spirals which range from open, barely perceptible coils to those which are so compressed that adjacent turns are in contact. The spirals may be dextrorse or sinistrorse and both direction and tightness of coiling are constant within species. The spores are more

Fig. 144.—Actinomycetes. Left, showing branched filaments with coiled terminal bodies which are fragmented into bacteria-like cells. Right, *A. antibioticus* showing both submerged mycelium and aerial sporulating mycelium. × 1200 (Starkey, Waksman and Woodruff).

resistant than the filaments and will survive 60° C. for as long as three hours, but are less resistant than bacterial spores. The tips of other filaments may become swollen and club-shaped. Segmentation of the filaments occurs, in some species as early as twenty-four hours while in others it is delayed three weeks or more; the segmented filaments break up to form bacillary bodies 4 to 6 μ in length which are morphologically indistinguishable from many true bacteria. In most smear preparations of the pathogenic forms, the filaments are broken up and the appearance is that of ordinary bacilli. All, or practically all, of the actinomycetes are gram-positive and some of the pathogenic forms are acid-fast; these last are immunologically related to the mycobacteria.

Both spores and fragments of mycelium grow in subculture. On semisolid media the growth is dry, tough and leathery, sometimes wrinkled, adherent to and piled above the medium; in many instances it resembles

that of the mycobacteria. In some cases the growth appears powdery or chalky owing to the formation of aerial mycelium. In liquid media growth occurs in the form of a dry, wrinkled surface film or, more often, as flakes or aggregates which adhere to the sides of the flask, especially at the surface, or sink to the bottom.

Pigment formation, with colors ranging over the entire spectrum, is common among the actinomycetes and differentiation is usually made between pigmentation of the vegetative mycelium and the spore-bearing aerial mycelium as well as pigment diffusing into the medium. Soluble purple and brown pigments are often observed on protein-containing media. The actinomycetes, especially the saprophytic forms, are physiologically active, utilizing a variety of carbon and nitrogen compounds, and many are actively proteolytic. An earthy to musty odor like that of freshly turned soil is produced by many species. The optimum temperature for growth is usually 20° to 30° C. though some of the pathogenic species grow at 37° C. and thermophilic species, analogous to thermophilic bacteria, are known. The great majority of actinomycetes are aerobic, but some of the pathogenic forms are anaerobic or at least must be cultivated under reduced oxygen tension.

Differentiation of the actinomycetes from one another is in part on a morphological and in part on a physiological basis, the latter including pigmentation and proteolytic activity. It has become increasingly apparent that there are two relatively well-defined morphological groups. The one, which includes all the pathogenic forms, is characterized by failure to form aerial mycelium and a marked tendency of the hyphae to segment and fragment into bacillary forms, often very early in the development of the culture. The other group is much larger, contains only saprophytic forms, and is characterized by the production of spores in aerial hyphae. There has been some tendency to separate the two by different generic names. The name *Nocardia* has been widely used, both for the entire group of actinomycetes and for the saprophytic forms only. Other names such as *Discomyces*, *Streptothrix*, *Cladothrix*, etc., have been proposed and used to a minor extent but are generally regarded as invalid for one reason or another. The name *Proactinomyces* was introduced by Jensen[4] in 1931 for the pathogenic actinomycetes and has gained some currency in recent years though it has not been generally accepted. The question has recently been considered by Waksman and Henrici[5] who separate the two groups by making them different families, the family *Actinomycetaceae* being retained for the pathogenic forms and including two genera, *Actinomyces* for the anaerobic forms, and *Nocardia* for the aerobic forms. A new family, *Streptomycetaceae*, is created for the saprophytes; questions of nomenclature and relationships of these forms need not be considered here.

The name *Actinomyces* will be used here for the pathogenic actinomycetes, all of which are characterized morphologically by failure to form aerial mycelium or spores. This group may be further subdivided on

[4] Jensen: Proc. Linnean Soc. N. S. Wales, 1931, *57*:364.
[5] Waksman and Henrici: Jour. Bact., 1943, *46*:337.

the basis of oxygen requirements and acid-fast staining properties. In addit on, a number of more or less diverse microorganisms are grouped with the actinomycetes by some workers. The so-called pleuropneumonia organisms (p. 495) are so considered by Ledingham[6] and certain of the

THE PATHOGENIC ACTINOMYCETES AND ACTINOMYCETE-LIKE FUNGI

Organism	Disease	Geographic Distribution
Actinomyces bovis	Actinomycosis in man, "lumpy jaw" in cattle	Ubiquitous; majority of cases reported in U. S.
Actinomyces madurae *Actinomyces bahiensis* *Actinomyces somaliensis*	Actinomycotic mycetoma in man	Tropics, including India and Egypt; few cases in U. S.
Actinomyces asteroides *Actinomyces gypsoides*	Deep-seated abscesses and pulmonary infection	Ubiquitous
Actinomyces keratolyticus	"Cracked heels"	India
Actinomyces minutissimus	Erythrasma	Ubiquitous
Actinomyces farcinicus *Actinomyces dermatonomus*	Animal parasites, do not infect man	Europe and Australia respectively
Actinomyces muris-ratti	Rat-bite, Haverhill fever	Europe and U. S., probably ubiquitous
Actinobacillus lignieresi	Actinobacillosis in cattle, very rare in man	South America, not in U. S.
Erysipelothrix rhusiopathiae	Natural parasite of hogs, erysipeloid in man	Europe and U. S., probably ubiquitous

non-sporulating obligate anaerobes (p. 489) are sometimes regarded as actinomycetes. Other microorganisms of uncertain position but showing affinities for the actinomycetes, including *Erysipelothrix*, the rat-bite fever fungus, and actinobacillus are discussed in this section.

Actinomyces Bovis

Though the term actinomycosis refers to any infection with an actinomycete, it is often thought of in connection with disease caused by *Actinomyces bovis*. This is the most clearly defined and best studied actinomycete infection and the disease occurs chiefly in cattle, and in few other animals; it is also occasionally seen in man. Although the disease was undoubtedly observed early in the nineteenth century, actinomycotic tumors being described by Leblanc[7] in 1826 under the name of osteosarcoma, it was first recognized as a specific parasitic disease by Bollinger[8] in 1877. At his instigation the fungus was studied by the botanist Harz[9] who described it and named it *Actinomyces* or ray fungus, because of the

[6] Ledingham: Jour. Path. & Bact., 1933, *37*:393.
[7] Leblanc: Jour. Méd. Veterin., 1826, p. 333.
[8] Bollinger: Deut. Ztschr. f. Tiermed., 1877, *3*:334.
[9] Harz: Deut. Ztschr. f. Tiermed., Suppl., 1878, *4*:125.

ray-like structure of its growth in the tissues, but did not cultivate it. In 1891 Wolff and Israel[10] isolated a microaerophilic actinomycete from pathologic material by anaerobic culture and in the same year an aerobic actinomycete was isolated by Bostroem[11] from similar sources and named *Actinomyces hominis* (sometimes called *Actinomyces graminis*). Bostroem's organism has been proved to have been a contaminant, however, and it is definitely established that the organism isolated by Wolff and Israel (called by some *Actinomyces israeli*) was that observed by Bollinger and Harz and the etiologic agent of the disease. These early observations were extended in large part through the work of Wright[12] and Emmons[13] and present knowledge has been summarized by Erikson.[14]

Morphology and Staining.—*Morphology in the Tissues.*—Actinomycosis is essentially a suppurative process, characterized by the formation

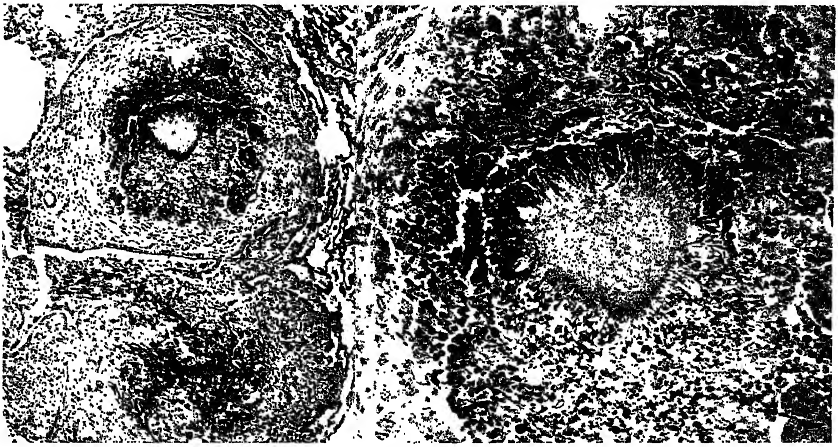


Fig. 145.—Actinomycotic (*A. bovis*) granule in rabbit lung. Hematoxylin and eosin. Left, two granules; × 80. Right, upper part of left field at higher magnification; × 300. Note the eosinophilic clubs at the periphery of the granule (Rosebury, Epps and Clark).

of granulation tissue, fistulation, sinus formation and by the presence in the pus of yellow granules ("sulfur granules"), the *Drüsen* of German writers. These are in fact colonies of the fungus and when examined microscopically are seen to consist of dense rosettes of club-shaped filaments in radial arrangement. The individual rosettes are usually 30 to 40 μ in diameter but sometimes are as large as 200 μ. The minute yellow granules, visible to the naked eye, may consist of a single rosette or may be made

[10] Wolff and Israel: Virchow's Arch. f. path. Anat., 1891, *126*:11.

[11] Bostroem: Beitr. Path. Anat. Allg. Path., 1891, *9*:1.

[12] Wright: Pub. Mass. Gen. Hosp., Boston. 1905; Jour. Med. Res., 1904–5, *8*:349.

[13] Emmons: Puerto Rico Jour. Pub. Health & Trop. Med., 1935, *11*:63; Pub. Health Repts., 1938, *53*:1967.

[14] Erikson: *Pathogenic Anaerobic Organisms of the Actinomyces Group.* Med. Res. Council (Gt. Britain) Spec. Rept. Ser. No. 240, 1940.

up of several. The rosette itself is made up of three kinds of structures: a central core of branching filaments, irregularly disposed but with a general radial arrangement; refringent, club-shaped bodies at the periphery radially arranged; and spherical coccus-like bodies. The granules may be crushed and examined in fresh preparations in which the clubs may be plainly seen or a stain such as eosin may be used which colors the sheath of the clubs. The filaments are gram-positive and the stain is useful for tissue sections; hematoxylin-eosin is quite satisfactory for sections. *A. bovis* is not acid-fast.

The filaments of the central core are branched, are often curved, sometimes spirally, and are thickly interlaced in a network of mycelium. The individual filaments have a granular appearance and, particularly in older granules, segmentation and fragmentation are common, giving the filaments the appearance of chains of cocci.

The club-shaped bodies at the margin of the granule are conspicuous

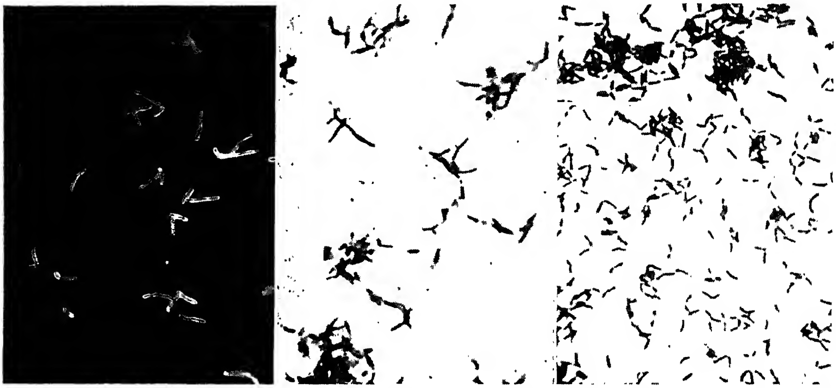


Fig. 146.—*A. bovis* from cultures. Left, darkfield × 900. Middle and right, gram stains of rough and smooth cultures respectively. × 1200 (Rosebury, Epps and Clark).

for their high refringency and general structureless, homogeneous appearance. They are pear-shaped swellings of the terminal ends of the filaments, and arise as distinct transformations of these. In young colonies the hyaline substance of which the clubs are composed is soft and may be dissolved in water, but as the age of the colony increases, the clubs become of firmer consistency. Their formation appears to be associated with the resistance of the tissues; when resistance to invasion is slight, they are absent, filaments alone being found. Clubs are, as a rule, more common in bovine than in human lesions.

The coccus-like bodies reported by various observers are probably of diverse nature. Such forms may result from the segmentation and fragmentation of filaments; in other cases they may be the ends of clubs appearing in the field of focus of the microscope. In still others they may perhaps be micrococci, secondary invaders of the suppurating actinomycotic lesion. (See p. 617.)

Morphology in Culture.—The colonial morphology of *A. bovis* grown anaerobically on semisolid media is sufficiently distinctive that, with experience, the colonies are readily differentiated from those of contaminating bacteria.[15] After four to six days' incubation the colonies are often less than 1 mm. in diameter. They are usually opaque, dead white or rarely showing a slight grey or yellow tinge, and have a pitted glistening surface and irregular margin. The growth, piled high above the surface of the medium, is similar to that of the tubercle bacillus. The colonies are tough and leathery, often coming away from the medium in one piece, and are difficult to emulsify. It has been observed that after continued cultivation the colonies tend to become smooth and less distinctive in appearance, and may be much softer in consistency. In shake cultures in dextrose agar the fungus grows below a depth of perhaps 10 mm. and appears as

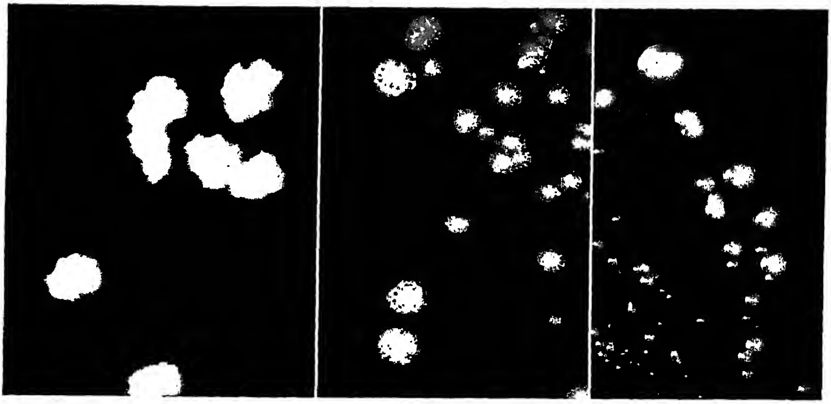


Fig. 147.—Colonies of *A. bovis* on brain heart infusion agar after six days' incubation. Left and center are rough types; × 3. The colonies on the right are the smooth type; × 6 (Rosebury, Epps and Clark).

irregularly shaped, opaque, whitish nodules which may reach a diameter of 2 to 3 mm. in a week. Stab cultures in dextrose agar give a dense grayish streak of small colonies along the lower part of the line of inoculation. In broth good growth usually occurs in the form of solid, whitish, mulberry-like granules at the bottom of the tube; the broth does not, as a rule, become cloudy.

The essential features of the rosette have been reproduced in cultures. The smaller colonies are rounded masses of branching and interlacing filaments. As the filaments become older they tend to fragment and the largest colonies are dense opaque masses of short filaments and rod forms. Clubs are not formed in the usual media but only in the presence of blood, serum or ascitic fluid and even there develop inconsistently. Stained smears of cultures show largely bacillary forms and a few fragments of branching filaments. The bacillary forms may be diptheroid in appearance, stain

[15] *Cf.* Rosebury, Epps and Clark: Jour. Inf. Dis., 1944, *74*:131.

irregularly, and some have terminal swellings; the last are quite unlike the peripheral clubs of the actinomycete granule.

Physiology.—Particular interest has centered about the relationship of *A. bovis* to oxygen. In the past it has been assumed that this microorganism is an obligate anaerobe and the identity of strains showing even scanty growth under aerobic condition has been questioned. It now seems to be established that it is microaerophilic with a limited oxygen tolerance, and some strains may grow sparsely in the air for one or two transfers. It is, however, best cultivated under anaerobic conditions in the presence of 5 to 10 per cent carbon dioxide. The limits in *p*H are similar to those of most bacteria and growth is best at 37° C. Growth is relatively slow even under optimum conditions and four to six days' incubation are usually required.

There appears to be no definite information regarding the nutritive requirements of the fungus; in general it does not appear to be fastidious in that reasonably good growth occurs in meat extract media with added dextrose (1 per cent). Rosebury, Epps and Clark[15] have found brain heart infusion agar most satisfactory and not improved by the addition of blood. Growth on potato is scanty and milk is not a good culture medium. It has been a general experience that *A. bovis* cultures are difficult to maintain in the laboratory and usually die out in spite of weekly or biweekly transfer. Erikson[14] has recommended alternate transfers on different media for the maintenance of cultures and Rosebury, Epps and Clark[15] report good results with this method. Glucose, maltose, lactose and salicin are fermented with acid and no gas, indol and hydrogen sulfide are not produced, and nitrate is actively reduced. These biochemical reactions have no differential significance.

Pathogenicity for Animals.—The majority of actinomycotic lesions in cattle are found in or about the head; the lower jaw and tongue in particular are affected, hence the common names of "lumpy jaw" and "wooden tongue" for this disease. In addition to the growth in the tongue and maxillary bones, actinomycotic lesions occur in the pharynx, lungs, skin, lymph glands and subcutaneous tissue, especially of the head and neck, and occasionally in other organs, notably the udder and liver. The growth of the parasite usually leads to the formation of a hard tumor which gradually increases in size and burrows into the adjacent tissues, softening and disintegrating the bony structure of the head, and at the same time forming new tissue, so that great distortion often ensues. The extension of the disease takes place by gradual invasion of the contiguous tissues, metastases being uncommon. When death occurs it is not, as a rule, due to any toxic effect but wholly to the mechanical action of the tumor in pressing upon or occluding the respiratory passages or in interfering with the taking or mastication of food. Generalized actinomycosis is rare. When it occurs the bloodstream rather than the lymph seems to be the channel by which the disease is spread. Secondary abscesses are found mainly in the liver.

The characteristic yellow granules or "*Drüsen*" are found in the suppurating mass of the tumor; if the pus is shaken up with water in a test

tube the small granules become evident and sink to the bottom of the tube. These structures are so typical that their demonstration by microscopic examination in a case of obscure suppuration is sufficient to establish a diagnosis. The new growth—granulation tissue—consists chiefly of epithelioid and spindle-shaped connective-tissue cells; small giant cells are sometimes present. To the naked eye, actinomycotic lesions in the lung and udder often resemble tuberculous nodules and at times have undoubtedly been mistaken for them; microscopic examination suffices to establish their nature.

The incidence of actinomycosis in cattle is uncertain, in part because it is readily confused with actinobacillosis; possibly something under 1 per cent is a fair estimate. Of the other domestic animals swine are most frequently affected, usually with primary lesions in the mammary glands. Horses and sheep are affected less often and the disease seldom occurs in dogs and is rare in cats.[16]

Experimental Animals.—Attempts to infect experimental animals with *A. bovis* have in general been disappointing in that only a small proportion of inoculated animals develop the disease and the lesions are limited and benign. In the last few years Henrici and his associates[17] have found that repeated inoculations are much more effective; Slack[18] was able to produce chronic progressive actinomycosis in this way in four of five rabbits. Rosebury, Epps and Clark[15] were less successful, and by repeated inoculation of twenty-four rabbits and sixteen guinea pigs produced infection in only five animals, progressive and fatal in two guinea pigs and one rabbit and localized and benign in two rabbits. In the experimental disease, however, the essential features of the natural infection are observed including the formation of tubercle-like nodules and the development of structurally typical granules with clubs.

Pathogenicity for Man.—Infection with *A. bovis* is observed in man from time to time, but it is not common. Sanford[19] found about 700 reported cases in the United States up to 1923; a large proportion were in the upper Mississippi Valley, a distribution which probably represents recognition rather than incidence of the disease.

The disease in man differs in minor respects from that in cattle. Actinomycotic infections of the bone are relatively less frequent, the disease being confined to the softer parts in most cases. There is usually a lesser production of new tissue and a more extensive softening and suppuration. The disease falls into three clinical types. About 60 per cent of the infections are *cervico-facial* and this type is often associated with dental defects or accidents and is a chronic, localized form of the disease which is relatively benign and usually susceptible to treatment. Some 14 per cent of the cases are *thoracic* infections and 8 to 18 per cent are *abdominal* in which the primary lesion is often in the appendix; in these types prognosis

[16] Martin: Univ. Pennsylvania Vet. Ext. Quart., 1942, *87:*15.
[17] Mathieson, Harrison, Hammond and Henrici: Amer. Jour. Hyg., 1935, *21:*405.
[18] Slack: Jour. Bact., 1942, *43:*193.
[19] Sanford: Jour. Amer. Med. Assn., 1923, *81:*655.

is poor.[20] Draining sinuses are usually found in all types and abscesses are frequently observed in the liver at autopsy. Generalization by hematogenous spread is occasionally seen and is relatively more common in man than in cattle. Meningitis and endocarditis have been reported. The characteristic granules with clubs are usually found in the pus but may be absent occasionally in draining sunuses, especially when the infection has spread rapidly, particularly in meningitis and empyema. The disease may terminate fatally in a few weeks through secondary infection or formation of emboli, or it may drag along in a chronic form for many years; spontaneous healing has been observed.

Epidemiology.—Direct transmission of *A. bovis* from one animal to another or from cattle to man has never been satisfactorily established. Many of the cases of actinomycosis reported in man are among persons

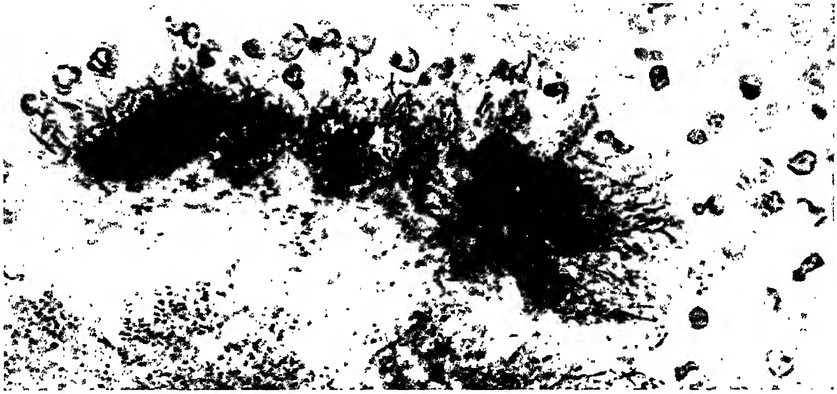


Fig. 148.—Pulmonary infection in man with *Actinomyces bovis*. Margin of a granule in lung tissue showing the ray-like structure. Note the bacteria designated *Actinobacillus actinomycetem-comitans* in the center of the granule. Gram stain; × 1000 (Humphreys).

who have not been engaged in agricultural pursuits and, so far as discovered, have not come in contact with any preexisting cases in animals or man. Nor has *A. bovis* been found to exist as a saprophyte in nature. There has been some confusion on this point since Bostroem's aerobic actinomycete is a widely distributed saprophyte; the Wolff-Israel organism, however, appears to be a strict parasite and as such is widely distributed in the normal human mouth and throat. Actinomycetes make up a large part of dental plaque material (p. 486) and fungi indistinguishable from *A. bovis* and capable of producing experimental actinomycosis in animals have been found in the absence of actinomycosis in carious teeth, dental and gingival scrapings and tonsillar crypts by many workers.[21] In fact it was early predicted by Wright[12] that *A. bovis* would be found to be a normal inhabitant of the mouth. In the light of present knowledge it

[20] *Cf.* Sanford and Voelker: Arch. Surg., 1925, *11:*809. Good: Arch. Surg., 1930, *21:*786.
[21] *Cf.* Lord and Trevett: Jour. Inf. Dis., 1936, *58:*115. Emmons: Puerto Rico Jour. Pub. Health & Trop. Med., 1935, *11:*63. Also Slack: *loc. cit.* and Rosebury *et al.*: *loc. cit.*

seems highly probable that the infection is endogenous in origin and that the fungus, normally present but of low pathogenicity, multiplies in the favorable environment provided by necrotic tissue resulting from injury, and occasionally establishes a focus of infection, possibly in association with bacteria which are eliminated as the infection progresses.

Immunity.—There appears to be little effective acquired immunity to *A. bovis* infection. Recently the possibility of the development of a hypersensitivity to the actinomycete cell substance has received some attention. The apparently greater efficacy of repeated small doses over a single massive inoculation in the experimental production of the disease has provided the basis for Henrici's[2, 17] suggestion that infection may depend on previous sensitization. This is not established as yet.

Isolation and Diagnosis.—As indicated earlier, the demonstration of the typical actinomycete rosette in the tissue or pus of a specimen is sufficient to establish a diagnosis of actinomycosis in man. Actinomycosis in cattle may be confused with actinobacillosis; the two are readily differentiated by examination of a gram-stained smear for the presence of gram-positive diphtheroid-like fragments of actinomyces filaments, or of gram-negative actinobacilli. As may be inferred from the foregoing discussion, animal inoculation is useless as a diagnostic method. Cultivation of the fungus is often, though not always, successful.

The procedure recommended by Wright[12] for the isolation of *A. bovis* in culture has been widely used. Granules, preferably from a closed lesion, are thoroughly washed in several changes of sterile water or broth, and then crushed between sterile glass slides. The material is inoculated into liquid (40° C.) 1 per cent dextrose agar in test tubes filled to a depth of 6 to 8 cm. After incubation at 37° C. for four to eight days, characteristic colonies develop, in greatest numbers in a narrow zone 5 to 12 mm. below the surface. These may be isolated from the shake culture in the usual way, washed in sterile water or broth if there is reason to suspect bacterial contamination and subcultured in deep tubes of dextrose agar. Even in the absence of contamination the method is not particularly satisfactory and frequently fails.

Rosebury, Epps and Clark[15] have shown that abundant and characteristic growth occurs on the surface of enriched agar media containing dextrose and incubated under anaerobic conditions in the presence of carbon dioxide. The fungus can, therefore, be readily isolated by the usual bacteriological procedures. They recommend brain heart infusion containing 2 per cent agar. The granule, exudate, or other material is streaked serially on plates of the medium by means of a sterile bent glass rod and the plates incubated four to six days under anaerobic conditions in the presence of 5 per cent carbon dioxide. By this method *A. bovis* can be isolated even in the presence of heavy bacterial contamination.

Mycetoma

Mycetoma (fungus tumor) or Madura foot is an affection occurring with some frequency in Southern India; the first cases were reported (1842 *et seq.*) from Madura, hence the name. It is a disease of tropical climates

though a number of cases have been observed about the Mediterranean in North Africa, in Greece and Italy. It also occurs in South America and the Caribbean islands. It has been observed in temperate climates, a few cases having been reported from Europe and the United States. It was first observed in this country by Wright[22] in Boston in 1898 and according to Gammel[23] twenty-four cases had been found in this country up to 1927. The disease was first studied systematically by Vandyke Carter and the earlier work is discussed at length by Chalmers and Archibald[24] who proposed the system of classification of the mycetomas which is generally used.

The disease usually affects the foot; occasionally the hand is attacked, and other parts of the body more rarely. It commonly occurs in those habitually going barefoot and the fungus presumably enters the body through some injury or abrasion. There is some evidence that it may remain latent for some time, even a period of years, and become active following injury. The part first involved, usually the sole of the foot, shows a small subcutaneous swelling which slowly enlarges and softens to become phlegmonous. It ruptures to the surface, sinus tracts form and the process burrows into the deeper tissues, producing swelling and distortion of the foot; the bones may or may not be extensively involved. Numerous small eminences are found on the surface, each the orifice of a sinus. The discharge is a viscid, slightly purulent, often foul-smelling fluid containing minute granular particles up to 1 mm. in diameter. The presence of these grains separates the mycetomas from pseudomycetomatous conditions observed in yaws, sporotrichosis, etc. The disease is strictly local, secondary abscesses seldom if ever occur and the internal organs are probably never affected. Iodide therapy is said to be effective in the actinomycete infections, but with extreme tissue destruction or other etiologic agents, surgical removal of the infected tissues, usually involving amputation, is necessary and curative.

The most obvious clinical difference in the mycetomas is the color of the grains present in the discharge. These may be white, yellow or black, and red grains have been observed. These grains, or colonies, are composed of mycelial filaments of the fungus; clubs are often present in the actinomycete grains, and chlamydospores in those of other fungi; and the color is due to the formation of pigment by the fungus. Differentiation into white or yellow grained types on the one hand, and black on the other, is clinically attractive but unsound since grains of the same color may be produced by widely different fungi, and in one instance both black and white grained types have been produced experimentally with the same strain of microorganism. It is customary, therefore, to divide the mycetomas into two etiologic types:—

(1) Actinomycosis—mycetoma of actinomycete etiology.

(2) Maduromycosis—mycetoma caused by molds and mold-like organisms.

[22] Wright: Jour. Exp. Med., 1898, *3*:422.
[23] Gammel: Arch. Dermat. and Syph., 1927, *15*:241.
[24] Chalmers and Archibald: Ann. Trop. Med. and Parasit., 1916, *10*:169.

It would seem that since none of the reported actinomycotic mycetomas in which the fungus was identified showed black grains, and black grains are associated with maduromycosis, the white or yellow grained type may be either actinomycosis or maduromycosis while the black grained type is probably maduromycosis. There is in general a greater tendency to bone involvement in the actinomycete infections. Otherwise there appear to be no clinical differences between the two types. The majority of reported cases are maduromycosis, but in this country eighteen of the twenty-four cases discussed by Gammel[23] were actinomycosis.

Actinomycotic Mycetoma.—A number of species of actinomycetes have been described in mycetoma; Gammel[23] lists thirteen and more recently Dodge[1] gives fifteen. Of these two appear to be the most important, the others having been observed only occasionally. *Actinomyces madurae* is widely distributed and has been found in Tunis, Egypt, Algiers and Greece, in Senegal, Somaliland and Abyssinia, in India and in South

Fig. 149.—*Actinomyces asteroides*. Smear from a pure culture. Gram stain; × 1050.

America and the Caribbean. *Actinomyces asteroides* was found in mycetoma in the Philippines and has since been observed in South America and in Europe. Of the others *Actinomyces bahiensis* has been found several times in Khartoum and *Actinomyces somaliensis* in Africa. It is of interest that *A. bovis* has been found in actinomycotic mycetoma in South America.

Maduromycosis.—The etiology of maduromycosis is much more heterogeneous, the causative organisms including molds and mold-like fungi which more properly belong in the next section. Of the molds, species of *Aspergillus* and *Penicillium* have occasionally been found in maduromycosis. The other etiologic agents include organisms of the genus *Madurella*, secreting a black pigment and producing a black grain mycetoma; there are eight species of which the first to be described, *Madurella mycetomi*, is the most common. Closely related forms producing no pigment and causing white grain mycetoma are three species of the genus *Indiella*; these are not common. One of the sporotrichia, *Glenospora*

(*Aleurisma*), is responsible for some of the black grain and white grain mycetomas and certain species of *Monosporium* (*Scedosporium*) cause both black and white grain mycetomas.

Diagnosis.—As indicated above, the mycetomas are characterized by the presence of granules in the discharge. The grains may be very hard and are usually treated with strong (20 per cent) sodium hydroxide to dissolve pigment and debris and examined in a wet, unstained condition for the presence of tangled mycelial filaments. Clubs may be observed in the actinomycete infections and thallospores, heavy-walled homogeneous structures, are found in *Madurella* and other infections. For differentiation and identification of the fungus, culture is necessary, on enriched dextrose agar in the case of actinomycetes and on Sabouraud's medium in the case of others.

Other Actinomycetes

Scattered cases of various types of infection with actinomycetes are occasionally reported. *Actinomyces asteroides*, originally found in a brain

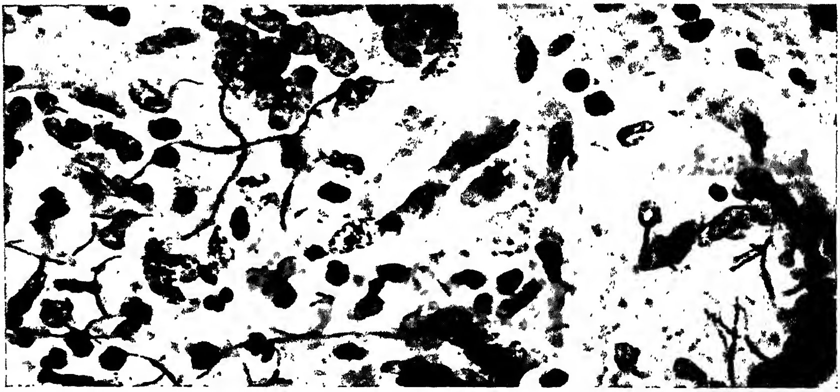


Fig. 150.—Bronchopulmonary actinomycosis in man resulting from infection with the non-ray type (aerobic) of actinomyces. Note the branching mycelium in the giant cells. Gram stain; × 860 (Humphreys).

abscess by Eppinger[25] in 1890, has been observed in a variety of pathologic processes including pulmonary affections sometimes called "pseudotuberculosis." Its occasional occurrence in mycetoma has been noted above. Both this organism and *Actinomyces gypsoides*, another strain of *A. asteroides*, isolated by Henrici and Gardner[26] from sputum in pulmonary disease, are acid-fast. These forms are immunologically related to the mycobacteria, the tubercle and leprosy bacilli.[27] A number of acid-fast actinomycetes have been isolated from similar affections and given various names; Henrici and Gardner[26] found twenty-six such cases in the literature to 1921. Infection occurs first in the peribronchial nodes, followed by the

[25] Eppinger: Ziegler's Beitr. z. path. Anat., 1890, *9*:287.
[26] Henrici and Gardner: Jour. Inf. Dis., 1921, *28*:232.
[27] *Cf.* Kedrowsky: Philippine Jour. Sci., 1937, *62*:439

development of a caseous bronchopneumonia with central softening and cavitation. The disease is of about six months' duration and may be confused with tuberculosis; in acid-fast stains of sputum smears only the bacillary forms of the actinomycete are present and cannot be distinguished from tubercle bacilli. It may be readily differentiated by culture and guinea pig inoculation.

A disease of the foot occurring in India, cracked heels or *keratodermia plantare sulcatum*, and known locally as *chaluni*, *haja*, or *panki*, has been shown by Acton and McGuire[28] to be caused by an actinomycete, *Actinomyces keratolyticus*. The fungus appears to have a lytic action on the horny layer of the epidermis and the disease is characterized by solution of the thick horny skin of the plantar surface in grooves. The skin cracks along these grooves to form deep fissures on the heel and in the thick sodden skin between the toes which extend through the corium to the subcutaneous tissues. Secondary infection, especially with streptococci, is common.

The disease *ulcus interdigitale* appears to be caused at times by the same organism. Deep fissures develop between the toes and large chronic ulcers appear which show little discharge and are very painful. The lesions may extend deeply into the interdigital cleft and secondary infection may necessitate amputation of the toe. The ulcers may also develop in the sole of the foot in the region of the heel and great toe. Infection with *A. keratolyticus* occurs during the monsoon season and appears to be contracted through walking barefoot on damp soil contaminated with manure.

Erythrasma, a superficial mycosis resembling tinea versicolor (p. 647), is caused by *Actinomyces minutissimus* (*Microsporon minutissimum*). The initial lesion is a small scaly macule which slowly enlarges to produce a yellowish to reddish brown scaly patch with reddened borders. The disease is usually confined to the groins. The axillae, the intergluteal cleft or other intertriginous areas are less frequently involved. It is never found on free (non-intertriginous) surfaces.

Other actinomycete infections take a variety of forms. Abscesses in various parts of the body, including the brain, are observed from time to time and a number of cases of corneal infection and conjunctivitis have been reported.

Actinomycotic infections of domestic animals are occasionally observed. One of the first to be reported was a farcy-like disease of cattle caused by *Actinomyces farcinicus*. Other infections are seen in sheep and goats in a variety of clinical forms. A skin disease of sheep of some economic importance because of the copious exudate which prevents shearing and makes the wool unmarketable, and due to *Actinomyces dermatonomus*, has been reported from Australia.[29]

When actinomycetes are isolated from some pathologic process and studied, it is often found that the fungus does not conform precisely to previously described species, and a new species is created. Many species have been described, therefore, of which a large proportion are of doubtful

[28] Acton and McGuire: Indian Med. Gaz., 1931, *66*:65.
[29] Bull: Australian Jour. Exp. Biol. and Med. Sci., 1929, *6*:301.

importance either because of the rarity of their occurrence, as having been observed only once, or because there was some doubt as to the etiologic relation of the fungus to the disease. Dodge[1] lists 140 species of actinomycetes of proved or suspected pathogenicity, including 29 which he regards as inadequately described.

ACTINOMYCES MURIS-RATTI

(RAT-BITE FEVER, HAVERHILL FEVER)

A microorganism variously known as *Streptothrix muris ratti*, *Haverhillia multiformis*, *Streptobacillus moniliformis*, *Actinomyces muris* and *Actinomyces muris-ratti* is the cause of an acute febrile disease sometimes given the descriptive name erythema multiforme. It is also called rat-bite fever because it may be acquired through the bite of infected rats.

For a good many years the etiology of the disease known as rat-bite fever was confused, for Schottmüller,[30] Blake[31] and others isolated from the blood of patients a fungus to which the name *Streptothrix muris ratti* was given, while Japanese work indicated that a similar disease, known in Japan as *sodoku*, is caused by a spirochete. In 1925 an epidemic, milk-borne disease with unusual, and distinctive clinical features occurred in Haverhill, Massachusetts, and was given the descriptive name *erythema arthriticum epidemicum* or Haverhill fever by Place, Sutton and Willner.[32] The causative microorganism was isolated by Parker and Hudson;[33] it proved to be a member of the *Actinomycetaceae* and was given the provisional name *Haverhillia multiformis*. In the same year Levaditi[34] independently studied a similar organism from a case clinically like Haverhill fever and named it *Streptobacillus moniliformis*. Other work has yielded similar results, and it is highly probable that the fungus isolated and studied by all these workers is the same, an actinomycete for which the proper name is *Actinomyces muris-ratti*. The Japanese work has also been amply confirmed and it is now clear that there are two types of rat-bite fever, completely different in etiology. The actinomycete infection concerns us here; the spirochetal disease is considered elsewhere (p. 697).

The microorganism is, like the other actinomycetes, pleomorphic in culture with branching filaments, fragmented, bacillary and cocco-bacillary forms being found in stained smears. Staining may be irregular, and swollen and club-shaped cells are found. It has been suggested that this organism is related to the organisms of the pleuropneumonia group. (See p. 495.) It is not acid-fast and, in contrast to most actinomycetes, is gram-negative. It requires enriched media for growth; Parker and Hudson[33] found whole rabbit blood, allowed to clot and then inactivated, and broth containing serum or ascitic fluid excellent liquid media, and glycerine extract of potato mixed with infusion broth, enriched with egg yolk and coagulated by inspissation, the best solid medium. Löffler's serum medium is not par-

[30] Schottmüller: Derm. Wschr., 1914, *58:* suppl. p. 77.
[31] Blake: Jour. Exp. Med., 1916, *23:*39.
[32] Place, Sutton and Willner: Boston Med. & Surg. Jour., 1926, *194:*285.
[33] Parker and Hudson: Amer. Jour. Path., 1926, *2:*357.
[34] Levaditi: Presse Med., 1926, *34:*340.

ticularly satisfactory. Growth is markedly better under anaerobic conditions in the presence of added carbon dioxide than it is in the presence of air.

When the disease in man is contracted through a rat bite, the initial wound heals but after a week or ten days becomes inflamed and painful. Adenitis develops and is followed by toxic symptoms which are the first evidence of the disease acquired from milk. These include headache, chills, vomiting and general malaise. An eruption of morbilliform character appears, especially on the extremities, and there is a multiple arthritis, often severe. The clinical resemblance to rat-bite fever of spirochetal etiology is very close, though Allbritten, Sheely and Jeffers[35] believe the two can be differentiated. The causative microorganism may be isolated by blood culture and has been found in the fluid of affected joints. Agglutinins are formed and according to Brown and Nunemaker[36] are of considerable diagnostic utility.

A. muris-ratti has been found to be a normal parasite of rats and mice and is present in the nasopharynx. It may assume pathogenicity for the rodent host and produce sporadic or epidemic disease of either an acute septicemic or chronic polyarthritic type.[37] An epizootic in wild mice of the latter type has been reported from Australia.[38] It has also been found in bronchopneumonia of rats.[39] It is clear that man may acquire the disease by the bite of normal rats and probably almost all sporadic cases arise in this way. Brown and Nunemaker[36] are of the opinion that rat bite produces the actinomycete infection much more commonly than the spirochete infection in this country. Farrell, Lordi and Vogel[40] report the fourteenth sporadic case of Haverhill fever on record, *i.e.*, in the thirteen years following its differentiation in 1926, and Brown and Nunemaker[36] have reported three additional cases; the number of undiagnosed cases and the proportion of previously reported cases of rat-bite fever that were *A. muris-ratti* infections are not known (*cf.* p. 698). As indicated above, the Haverhill epidemic was milk-borne; another milk-borne outbreak involving eighty-six persons was reported[41] in 1934. Parker and Hudson[33] found evidence which suggested that the Haverhill milk was contaminated by an infected cow but this could not be proven; others have suggested contamination of milk by rats as a possibility.

Actinobacillus

Actinobacillus Lignieresi.—In 1902 Ligniers and Spitz[42] isolated a non-motile, non-branching, non-acid-fast, gram-negative bacillus from the

[35] Allbritten, Sheely and Jeffers: Jour. Amer. Med. Assn., 1940, *114*:2360.

[36] Brown and Nunemaker: Bull. Johns Hopkins Hosp., 1942, *70*:201.

[37] Levaditi, Selbie and Schoen: Ann. Inst. Pasteur, 1932, *48*:308. See also Mackie, van Rooyen and Gilroy: Brit. Jour. Exp. Path., 1933, *14*:132 and Strangeways: Jour. Path. Bact., 1933, *37*:45.

[38] Williams: Med. Jour. Australia. 1941, *1*:357.

[39] Tunnicliff: Jour. Inf. Dis., 1916, *19*:767.

[40] Farrell, Lordi and Vogel: Arch. Int. Med., 1939, *64*:1.

[41] Place and Sutton: Arch. Int. Med., 1934, *54*:659.

[42] Ligniers and Spitz: Bull. Soc. Cent. Med. Vet., 1902, *20*:487, 546.

lesions of a disease of cattle which closely resembles actinomycosis and with which it is frequently confused. The microorganism is known as *Actinobacillus lignieresi* and the disease as actinobacillosis. Originally found in Argentina, it appears to be relatively common and has been recognized in Europe and in this country.[43]

Granules, very similar to but smaller and more numerous than the "sulfur granules" of actinomycosis, are found in the thick pus from the lesions. These granules or colonies contain clubs radially arranged about a center which is composed of detritus and gram-negative bacilli. The microorganism is pleomorphic in culture; diplococci and slender rods are found in smears from cultures in liquid media while long curved forms are present in deep colonies in agar media. The bacilli are 0.4 μ in diameter and 1 to 15 μ in length.

Surface colonies on laboratory media are small, 0.5 to 1 mm. in diameter, smooth, glistening, convex, bluish white and delicate in appearance. In liquid media, such as serum-glucose-broth, the growth consists of small grayish granules adhering to the sides of the tube and readily broken loose by shaking; broth does not become turbid. The microorganism appears to be a strict parasite and grows very poorly or not at all except in media containing serum or whole blood. It is an obligate aerobe though primary cultures are often more successful in fluid media or serum-glucose-agar stab cultures, especially when incubated in an atmosphere containing 10 per cent carbon dioxide. Cultures grow up in twenty-four hours at 37° C.; growth is very slight at 20° C. Dextrose, lactose, sucrose, maltose, raffinose and mannitol are fermented; the fermentation of xylose is irregular. Arabinose, dulcitol, salicin and inulin are not fermented. Very small amounts of indol are produced, coagulated serum is not liquefied, and litmus milk is usually unchanged but sometimes slightly acid.[44] The systematic position of *Actinobacillus* is uncertain. Thompson[45] has grouped it with the glanders bacillus and Bergey (1939) placed it in the family *Bacteriaceae*. It is generally regarded, however, as more closely related to the actinomycetes.

As indicated above, the disease in cattle closely resembles actinomycosis, differing in that the bones are seldom affected and the lesions are found in the soft tissues, the regional lymphatics being commonly involved; the subcutaneous tumors break down in time to form abscesses. The subcutaneous lesions respond to surgery but infection of the tongue, "wooden tongue," is often fatal. The disease has been observed to occur in both epizootic and sporadic form. It is readily reproduced by inoculation of cattle but the microorganism is only feebly pathogenic for the usual experimental animals; massive intraperitoneal inoculation of the guinea pig produces a scrotal reaction similar to the Straus reaction (p. 547). A very few probable cases of human infection have been reported.

[43] *Cf.* Vawter: Cornell Vet., 1933, *23*:126.

[44] There is some discrepancy in the literature on these points, including primary isolation culture, suitability of coagulated serum media, etc. Here the writer has followed Hagan: *The Infectious Diseases of Domestic Animals.* Comstock Publishing Co. Ithaca, 1943.

[45] Thompson: Jour. Bact., 1933, *26*:221.

Actinobacillus Actinoides.—This microorganism was originally isolated from the lungs of calves suffering from a chronic pneumonia[46] and later from a similar affection in white rats.[47] It is not pathogenic for other experimental animals and in fact its etiological relationship to the observed disease is open to some question.

Actinobacillus Actinomycetum-Comitans.—This bacillus has been observed and isolated from human cases of infection with *Actinomyces bovis*, first by Kligler[48] in Germany, and later by Comstock[49] in England and Bayne-Jones[50] in this country. It occurs as densely packed gram-negative cocco-bacilli in contrast to the gram-positive actinomycete mycelium in the interior of the sulfur granules (see Fig. 148). It has never been reported in bovine actinomycosis and the significance of its presence is open to question.

Erysipelothrix Rhusiopathiae

Microorganisms closely related to the actinomycetes have been found to be the causative agents of swine erysipelas and a type of mouse septicemia; they also infect man and the disease produced is termed erysipeloid to distinguish it from erysiplas of streptococcal etiology (p. 329). It was thought for a time that the mouse septicemia organism isolated by Koch,[51] the organism isolated from swine by Pasteur and Thuillier[52] and by Löffler,[53] and that found by Rosenbach[54] in human erysipeloid were distinct species of a genus to which the name *Erysipelothrix* has been given, and they were called *Erysipelothrix muriseptica*, *Erysipelothrix rhusiopathiae*, and *Erysipelothrix erysipeloides* respectively. The first of these, sometimes called *Bacterium murisepticum*, is not to be confused with *Pasteurella muriseptica* (p. 447) which also has the same synonym. Other names that have been used are *Erysipelothrix rhusiopathiae suis*, *Erysipelothrix porci*, *Bacillus erysipelatis suis*, *Actinomyces rhusiopathiae*, and the swine *rotlauf* bacillus. It is now generally agreed that these organisms are identical, or at least closely related varieties of the same species, for while their morphology is variable they are immunologically identical, and are recognized as a single species, *Erysipelothrix rhusiopathiae*.

Morphology and Physiology.—*Ery. rhusiopathiae* occurs in two rather well-defined morphological types usually designated smooth and rough though their relationship to the S and R variants of bacterial dissociation is not clear. The smooth type appears as a small, slender, sometimes slightly curved, non-motile, non-sporulating, gram-positive rod. Long chains of bacilli and filaments, sometimes beaded and showing swollen areas, are present in smears of the rough form. Both stain readily

[46] Theobald Smith: Jour. Exp. Med., 1918, *28*:333.
[47] Jones: Jour. Exp. Med., 1922, *35*:361.
[48] Kligler: Centralbl. f. Bakt., I Abt. Orig., 1912, *62*:191.
[49] Comstock: Brit. Jour. Exp. Path., 1920, *1*:197.
[50] Bayne-Jones: Jour. Bact., 1925, *10*:569.
[51] *Cf.* Koch: *Investigations into the Etiology of Traumatic Infective Diseases.* New Sydenham Society. London. 1880.
[52] Pasteur and Thuillier: C. R. Acad. Sci., 1883, *97*:1163.
[53] Löffler: Arb. Kaiserl. Gesundshamte, 1886, *1*:46.
[54] Rosenbach: Ztschf. f. Hyg. u. Infektionskr., 1909, *58*:343.

and sometimes irregularly with deeply staining granules. Colonies of the smooth form on semisolid media are round, convex, amorphous, water-clear, and small, perhaps 0.1 mm. in diameter, and broth cultures are uniformly turbid. The rough form produces larger colonies with a granular, curled appearance like that of very small colonies of anthrax bacilli, while growth in liquid media is in the form of flocculent, hair-like masses with little or no turbidity. The most characteristic growth is in gelatin stab cultures; bead-like colonies appear along the line of inoculation from which lateral filamentous growth occurs, resembling a test tube brush.

The organism is microaerophilic but will grow under aerobic or anaerobic conditions; growth appears on the usual infusion media with twenty-four hours' incubation at the optimum temperature of 30° C. There is no growth on potato. Fermentation reactions are variable from strain to strain but most ferment dextrose, lactose and levulose. Nitrate is reduced

Fig. 151.—*Erysipelothrix rhusiopathiae*. Pure culture. Note the similarity of this microorganism to the actinomycetes. × 1000 (Kral).

and hydrogen sulfide is produced, but indol is not formed and litmus milk is unchanged or slightly acidified.[55]

Ery. rhusiopathiae is somewhat more than ordinarily resistant to drying and to various preservative processes such as smoking, pickling and salting and survives for relatively long periods of time in putrefying meat and in water. It is probably in part because of survival of the organisms in filth that infected areas experience recurrences of the disease year after year.

Pathogenicity.—In swine four clinical types of disease are found. In the *acute septicemic* form, with lesions in the viscera and internal organs, the case fatality rate is high, perhaps 80 per cent, with death in three to five days. The *urticarial* form, known as "diamonds" or "diamond skin disease" because of the occurrence of reddish to purplish rhomboidal blotches on the skin, may occur with or without visceral involvement and is seldom

[55] The cultural and biochemical characteristics of this organism are discussed in detail by Karlson and Merchant: Amer. Jour. Vet. Res., 1941, 2:5.

fatal. The *chronic* form is a vegetative endocarditis with erosion of the mitral valves in particular; death always results eventually. *Arthritis* may complicate the other clinical types or occur independently; it is usually not fatal but growth is stunted. The organisms are excreted in large numbers in the feces and it is generally assumed that natural infection takes place by mouth although experimental feeding has given irregular results. Infection is spread in part by healthy carriers as well as diseased animals and pork trimmings in garbage probably account for isolated cases. Swine erysipelas is of very great economic importance in Europe, especially in Germany where it is known as *Schweinerotlauf*, and in recent years has been found to be more important in this country, at least in certain areas, than formerly thought.

Sheep are occasionally infected with *Ery. rhusiopathiae* and develop a polyarthritic form of the disease. The organism is also pathogenic for a variety of birds and in this country turkeys are most often seriously affected;[56] cyanosis is a prominent clinical feature and evident in the "blue comb."

The disease may be reproduced by the inoculation of swine though the results are irregular. Of the usual laboratory animals, white mice and pigeons are highly susceptible, dying in one to four days after subcutaneous inoculation, and are commonly used in diagnosis. The rabbit is not very susceptible and the guinea pig is quite resistant. In general the virulence of the microorganism varies widely.

Pathogenicity for man.—Human infection with *Ery. rhusiopathiae* is well known.[57] The septicemic type with diffuse erythema is rare in man and only a very few instances have been reported. The chronic form with endocarditis and polyarthritis is also very rare. The usual type of infection is an erythematous-edematous lesion, the local lesion commonly developing on the fingers or hand from an abrasion where the infection enters. The lesion, although spreading, never extends beyond the wrist. There is some swelling and a marked erythema of the region and sometimes a local arthritis and regional adenitis. The disease is usually self-limiting and terminates within a month. The organism may be cultivated from an excised piece of skin from the lesion.

Human infection can almost invariably be traced to contact with animals and animal products such as meat, hides, bones and manure, or to fish and shellfish[58] and the disease is, therefore, associated with certain occupations. For example, more than half the cases found in the Philadelphia region were in slaughterhouse workers. The disease has been observed in workers in a bone button factory using cattle and hog bones,[59] and it is not uncommon in fishermen and fish handlers. It also occurs in persons working in kitchens with raw meat and fish. In this country con-

[56] The incidence and epidemiology of the disease are discussed by Rosenwald and Dickinson: Amer. Jour. Vet. Res., 1941, *2*:202.

[57] The human disease is discussed at length by Klauder: Jour. Amer. Med. Assn., 1938, *111*:1345. See also Everts: Jour. Amer. Vet. Med. Assn., 1941, *98*:213.

[58] Klauder and Harkins: Jour. Amer. Med. Assn., 1931, *96*:1205. Also Klauder: Jour. Industr. Hyg., 1932, *14*:222.

[59] McGinnes and Spindle: Amer. Jour. Pub. Health, 1934, *24*:32.

tact with live fish and crustacea appears to be the chief source of infection. Hettche[60] has reported finding *Ery. rhusiopathiae* in 10 of 30 specimens of sewage at Königsberg in Prussia and in 5 of 52 specimens at Münich; the source apparently was slaughterhouses where infected animals were being killed. Under experimental conditions the organisms not only survived for some days in water but fish developed a latent infection when fed infected meat; the microorganism could be isolated from most organs including the kidney, and were excreted in large numbers in the urine, contaminating the water.

Immunity.—There is a definite immune response in infected hogs as evidenced by the formation of agglutinins which have diagnostic value. Antiserum has therapeutic value and in man is given both intramuscularly and by infiltration about the local lesion. Passive immunization of swine is an effective prophylactic but the immunity lasts no longer than two weeks. Animals may be actively immunized with the vaccine developed by Pasteur and Thuillier[52] who attenuated the organism by passing it through rabbits. Vaccine erysipelas occurs with some frequency, however, and the method has now been largely superseded by the simultaneous inoculation of virulent culture and antiserum. Since the vaccine maintains and spreads infection in herds, the Bureau of Animal Industry has only recently (1942) permitted its limited use in this country and only in areas where the disease has become prevalent. Both methods provide adequate protection for eight to twelve months; periodic reimmunization is required for breeding stock.

II. THE MOLDS AND MOLD-LIKE FUNGI

The fungi proper, or *Eumycetes*, are quite distinct from the true bacteria, being closely related to the higher algae but differing from them in that they lack chlorophyl. Phylogenetically, their differentiation may have arisen through a degenerative loss of chlorophyl and adaption to a chemosynthetic type of metabolism. Some of the fungi are unicellular, others are multicellular, and still others are sometimes unicellular and sometimes multicellular, that is to say, dimorphic; some of the parisitic fungi are unicellular in the tissues and multicellular in culture. For present purposes we shall consider the multicellular forms here under the head of molds and mold-like fungi, and the unicellular forms such as yeasts and yeast-like fungi in the following section, though the occurrence of intermediate forms and taxonomic considerations makes such a differentiation inconsistent.

Hyphae and Mycelium.—The multicellular fungi are made up in large part of cells attached end to end to form filaments or *hyphae*. These are considerably larger in diameter than the actinomycete filaments described earlier, and the latter are perhaps to be regarded as a rudimentary type of hyphae. In some instances certain of the hyphae may be coiled to form helices. The mass of branching, intertwining and sometimes anastomosing hyphae making up a colony of the fungus is the *mycelium*. The entire mass is also sometimes called the *thallus*.

[60] Hettche: Arch. f. Hyg. u. Bakt., 1937, *119*:178.

Two main structural types of mycelium may be distinguished. In one of these the cells making up the hyphae are not separated by cross walls or septa, making possible a characteristic flowing of protoplasm through the multinucleate structure. Such a structure is said to be *non-septate* or *coenocytic*. Certain of the algae are of similar structure and the fungi thus characterized are called *phycomycetes* or algae-like fungi. In the majority of fungi, however, the hyphae are *septate*, each cell separated from the other by cross walls. The individual cells contain a single nucleus, as in the *ascomycetes*, or sometimes two as in certain of the *basidiomycetes*. Thus these three main classes of fungi may be distinguished in part on the basis of the structure of the mature, non-sporulating hyphae, though this is not one of the main characteristics upon which their differentiation is made.

The mycelium is further differentiated into two general types which differ in function. One of these, the *vegetative mycelium*, consists of masses of hyphae within the colony, adjacent to and growing into the substrate, and is concerned with the assimilation of food materials. Fragments of such mycelium will, of course, reproduce if transferred. The other type, or *reproductive mycelium*, usually extends into the air to form an *aerial mycelium* and gives rise to reproductive bodies or spores. The mode of spore formation and the structure of the spore and spore-bearing elements are the characteristics by which the fungi are differentiated, classified and identified.

It may be noted that in addition to sporulation and the vegetative growth of hyphae, many species of fungi reproduce by the separation of cells, known as *oidia*, from any part of the mycelium. These vegetative reproductive forms may give rise to new mycelium or reproduce themselves by budding like the yeasts, according to the environment in which they are placed.

Spore Formation.—Two kinds of spores are to be distinguished, the *sexual spores* which are produced by the fusion of two cells which may or may not differ morphologically and which are referred to as plus and minus rather than male and female, and the *asexual spores* which arise by differentiation of the cells of the spore-bearing hyphae without fusion. Fungi for which both sexual and asexual types of spore formation are known are "perfect fungi" and are further differentiated on the basis of differences in asexual spore formation and other morphological characteristics. This group includes the phycomycetes, the ascomycetes and the basidiomycetes.

Sexual Spore Formation.—The most common of the phycomycetes are species of *Mucor* and *Rhizopus*. These molds produce a sexual spore known as a *zygospore* by the fusion of neighboring filaments of the same or different plants. The ascomycetes, which include a number of genera as well as certain strains of *Penicillium* and of *Aspergillus*, and also the yeasts, form sexual spores known as *ascospores* because they are contained in an *ascus* or sac. Their formation is simplest in the yeasts in which two contiguous cells fuse by means of minute tube-like processes, the nuclei unite, and the resulting single nucleus divides three times to give eight daughter

nuclei. Reserve material accumulates about each nucleus, a spore wall is formed, and the cell containing this is the ascus. In most of the ascomycetes the process is somewhat more complex and the cells which fuse may be distinguishable and are termed the *öogonium* and *antheridium*. The cell resulting from fusion gives rise to new hyphae, the next to last cell is binucleate, and these nuclei fuse and then divide to form the ascospores, *i.e.*, two nuclear fusions are involved in the entire process. The basidiospores are also formed by a complex process including nuclear fusion; this group is made up of the mushrooms, puffballs and other fleshy fungi, none of which is pathogenic.

Fungi Imperfecti.—A large group of fungi, which includes practically all of the pathogenic forms, is made up of those for which a sexual phase has not been demonstrated and only asexual spores are produced. They

Fig. 152.—*Rhizopus* sp. Mounted in Annam's medium. Note the single-celled (nonseptate) mycelium and ruptured, empty sporangia. The small oval bodies are free spores. The root-like structure at the base of the hyphae is the "hold-fast" by which the mold is attached to the nutrient medium. × 80.

are known as the "imperfect fungi" or Fungi Imperfecti and are sometimes called *hyphomycetes*; they make up the fourth group of eumycetes. The group is necessarily only provisional and various species of fungi are removed from it from time to time as their sexual phases are discovered. It is also heterogeneous and includes species which are much more closely related to those of other groups than to one another; for example, certain species of *Aspergillus* are classed with the ascomycetes while others, whose sexual phase is as yet unknown, are placed with the Fungi Imperfecti.

Differential Characteristics of Genera and Species.—While sexual spore formation is the basis for primary differentiation of the eumycetes, genera and species of both the "perfect fungi" and the Fungi Imperfecti are distinguished by other morphological characteristics. These, however, assume primary practical importance, especially for the pathogenic forms. They may be summarized briefly:

(1) **Reproductive Structures.**—The characteristics of asexual spore formation:

(a) In the more primitive molds, the phycomycetes, the asexual spores are characteristically produced within a capsule or *sporangium*. For example, in *Rhizopus nigricans*, the common white cottony mold found on damp bread, horse dung, etc., erect unbranched hyphae arise from the single-celled mycelium and near the apex of each a septum forms. The tip then swells into the globular sporangium, within which numerous oval spores develop; the wall of the ripe sporangium ruptures easily, and the spores are discharged by the swelling of the gelatinous mass in which they are embedded (see Fig. 152).

(b) In the higher molds the asexual spores are not enclosed in a capsule, but are produced free by segmentation of the tips of the hyphae. Such spores are known as *conidia* and the spore-bearing hyphae as *conidiophores*. Various types of structures are developed. For example:

(i) In *Aspergillus* the unbranched conidiophore arises from an enlarged cell of the vegetative mycelium, designated the foot cell, and terminates in a swollen tip, the vesicle. A number of little, tenpin-shaped stalks, the *sterigmata* or *phialides*, are given off from the vesicle and the chains of conidia are abstricted from these (see Fig. 153).

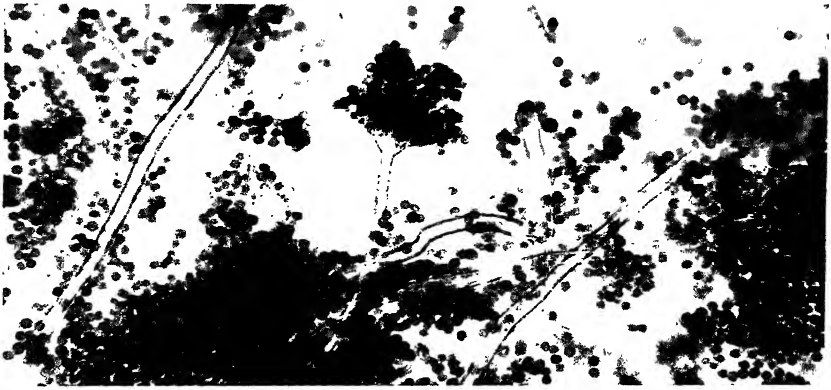


Fig. 153.—*Aspergillus* sp. mounted in Annam's medium. Note the fully developed sterigmata and the chains of conidia abstricted from the tips. The large dark masses are made up of free conidia. × 370.

(ii) The common blue-green mold, *Penicillium*, has a similar structure; the terminal portion of certain hyphae breaks up into finger-like verticillate branches and, on the tips of these, sterigmata abstrict chains of conidia (see Fig. 154).

(iii) In *Hormodendrum* the formation of conidia differs from that of many other molds. Instead of successive conidia being formed by repeated constriction of the tip of the sterigmata to form a chain in which the terminal spore is the oldest, the conidiophore forms but one spore which buds to form a second, the second forms a third, etc. Furthermore, a spore may develop more than one bud simultaneously thus producing branching chains of conidia. It may be noted that differentiation is made among these organisms on the basis of formation of phialides.

(iv) *Aleuriospores* resemble conidia in a general way in size and shape but differ in that they are formed on short lateral branches of hyphae, and sometimes directly on the hypha itself, rather than on the tips of specialized conidiophores, and are not set free when mature but are liberated only when the mycelium that forms them disintegrates. When the lateral branches are very short with many spores and/or the aleurospores are directly attached to the hyphae in considerable numbers, the structures are sometimes referred to as *hyphae sporiferae*, *thyrsi sporiferae*, or simply as *thyrsi* (see Fig. 163). Aleuriospores are perhaps to be regarded as lateral chlamydospores (see below) and are characteristic of the ringworm fungi in particular.

(v) *Spindle-spores* or *fuseaux* are large, thick-walled structures, septate and composed of several cells, which are formed by the aerial mycelium. Fuseaux are formed in large numbers by some species of fungi and only rarely by others and assume some differential significance, especially among the dermatophytes. Some workers regard shape, blunt or pointed ends, and other morphological characters of these bodies as valid differential characteristics (see Figs. 160 and 161).

(vi) Fragmentation of the mycelium results in the production of bodies termed *arthrospores*. These are often produced by the dermatophytes in the lesions in the skin and hair but are only rarely observed in culture. They are not true spores and are perhaps best regarded as oidia in structure and function though this point is seldom made.

(c) Large, thick-walled structures formed within the vegetative mycelium, sometimes within hyphae, at others laterally on short stalks, are designated *chlamydospores* (see Fig. 159). These structures are often irregular in form and some are composed of several cells and approach a spindle form. In such cases they resemble fuseaux and, in fact, in much of the literature are referred to as fuseaux.

(2) **Vegetative or Mycelial Structures.**—A number of structures are formed by the vegetative mycelium which have no reproductive significance but are of considerable value in the differentiation of the pathogenic fungi.

Fig. 154.—*Penicillium* sp. mounted in Annam's medium. Note the characteristic finger-like verticillate branches and the terminal chains of conidia. × 440.

(a) *Spirals* or coiled hyphae, similar to the coiled filaments of actinomycetes described in the preceding section, are observed in a number of the pathogenic mold-like fungi (see Figs. 162 and 164).

(b) *Nodular organs* are formed by some species. These are enlargements in the mycelium which consist of closely twisted hyphae, either side branches twining about the parent stem or different hyphae twisted together, and the resulting structure has a nobby, nodular appearance.

(c) *Raquet mycelium* or *mycelium en raquette* is a term applied to certain hyphae, usually larger than the others, that show a regular enlargement of one end of each segment, large and small ends being in apposition (see Fig. 165).

(d) The term *pectinate bodies* is applied to unilateral short irregular projections from the hyphae, giving a "broken comb" appearance.

(e) *Favic chandelier* is the name applied to the structure formed by the occurrence of numerous short multiple hyphal branches having the appearance of a reindeer horn. This structure is not common, occurring only in the favus fungus, *Achorion schoenleini* (see Fig. 166).

(3) **Colonial Morphology.**—The morphology of colonies on Sabouraud's "differen-

tial" medium has assumed greater importance for the dermatophytes than for the other pathogenic fungi. In fact, in this group primary differentiation is made on this basis and colonial morphology often serves to identify a species.

Microscopic Examination.—The methods used in the microscopic examination of the fungi vary somewhat according to the nature of the material and the purpose of the examination. In general the staining methods so useful in the study of the bacteria are not applicable; the fungi are all gram-positive and may be found in gram-stained smears but their morphology is obscured. Wet, unstained or lightly stained preparations are most informative.

Specimens.—Open or draining lesions are almost always so heavily contaminated by secondary bacterial invasion that fungi are very difficult to find; in material from surgically opened lesions they are usually demonstrable though not so numerous as bacteria in corresponding bacterial infections. In the dermatomycoses secondary bacterial infection ordinarily does not interfere with the microscopic demonstration of the fungus elements. The dermatophytes live in keratin material exclusively, and specimens should be taken from scrapings of horny layers, tops of vesicles, scrapings from nail plate, and hair.

The material should be mounted in strong (10 to 20 per cent) hot sodium hydroxide, which dissolves or makes translucent the tissue elements but ordinarily does not affect the fungi, and examined as a wet unstained preparation. Antiformin or lacto-phenol may be used instead of sodium hydroxide. Care must be taken to distinguish between spores and fat globules and between mycelium and fibrin strands; a mycelial-like structure ("mosaic fungus") may be formed in some preparations, presumably from cholesterol. Fungi may be demonstrated in tissue sections, as the walls of abscesses, granulomatous tissue, etc., by the Unna-Pappenheim (methyl green-pyronin) stain.

Cultures.—A bit of growth is removed from the colony, teased apart in a drop of water, and examined as a wet preparation. While the various structures may be seen, the arrangement of the elements is seriously disturbed. Slide cultures, such as those described by Henrici,[1] show the structure and arrangement of the growth and may be made into permanent mounts.

Cultivation.—Most of the fungi grow very rapidly but the pathogenic forms usually grow relatively slowly and two or three weeks' incubation may be necessary. Their morphology is markedly affected by the type of medium on which they are grown. In general they show no unusual nutritive requirements and grow readily on all the usual bacteriological media, especially if a sugar is added. Many molds tolerate a high acidity and may be cultivated on tartaric acid-dextrose nutrient agar on which bacterial growth is inhibited. Most grow well on modified Czapek-Dox medium (a glucose-nitrate synthetic medium) which is reproducible and has been widely used as a "standard" agar for descriptive purposes. Sabouraud's medium, a peptone-maltose agar, is perhaps the most widely used medium in medical mycology for the isolation and maintenance of cultures, especiallv the dermatophytes. Because of the influence of the com-

position of the medium on morphology, it is insisted that only "crude maltose of Chanut" (4 per cent) and "granulated peptone of Chassaing," brands obtainable only from a certain firm in Paris, be used. This is Sabouraud's "proof agar" (milieu d'épreuve). For most work, however, any maltose or peptone suffices and crude dextrose, such as corn syrup, may be substituted for the maltose.

PATHOGENIC MOLDS AND MOLD-LIKE FUNGI

Organism	Disease	Geographic Distribution
Aspergillus fumigatus	Aspergillosis—pulmonary in birds, otomycosis and pulmonary infection in man	Europe and U. S.; otomycosis most prevalent in India, probably ubiquitous
Fonsecaea pedrosoi	Chromoblastomycosis	Ubiquitous with the possible exception of Europe; especially South America and Puerto Rico
Sporotrichum schencki	Sporotrichosis	Europe and U. S., probably ubiquitous in temperate climates
Microsporum species *Trichophyton* species *Achorion* species *Epidermophyton* species *Endodermophyton* species	Dermatophytosis in man, some species natural parasites of animals	In general ubiquitous, variable from species to species

Aspergillus (Aspergillosis)

The blue-green mold commonly observed on damp bread and similar materials is usually an aspergillus, often called *Aspergillus glaucus*, though this name has been so indiscriminately applied that it now has little or no precise significance. So far as is known, these bread molds are not pathogenic. A similar mold, however, also producing a blue-green pigment and known as *Aspergillus fumigatus*, is highly pathogenic for birds and also occasionally for man.

Pathogenicity for Animals.—Aspergillosis of domesticated birds, pigeons, ducks and chickens, is not uncommon and at times assumes economic importance. Three types of infection occur, infection of the air sacs, and both nodular and pneumonic forms of lung infection. Infection of the air sacs takes the form of a superficial infection of the epithelial lining, which becomes thickened and covered with a mat of green, sporulating mycelium. In the nodular form of the disease tubercle-like masses of infiltrated tissue, necrotic in the center, are formed. A diffuse infiltration develops in the pneumonic form and the lung tissue is consolidated and grayish white in color. The pneumonic disease sometimes assumes epidemic form in chicks and is known as "brooder pneumonia." The source of infection is usually moldy grain or straw. The fungus may also invade the egg during incubation with infection of the embryo. Cattle, sheep, and especially horses develop aspergillosis though less commonly

than birds; the lesions are pulmonary and may be either nodular or pneumonic.

Strains vary widely in their pathogenicity when tested by inoculation of experimental animals. In general those isolated from infections are highly virulent, while those found in air and elsewhere are of low virulence. With virulent strains a fulminating, rapidly fatal pneumonia may be produced in pigeons by inhalation of spores, while feeding grain overgrown with the mold often produces an infection of the air sacs. Intravenous inoculation of pigeons results in an acute infection with multiple miliary abscesses, especially in the lungs, if the dose is not too large. Rabbits inoculated intravenously with spore suspensions of virulent strains usually die in three to five days with multiple abscess formation, notably in the kidney, while subcutaneous or intraperitoneal inoculation produces localized

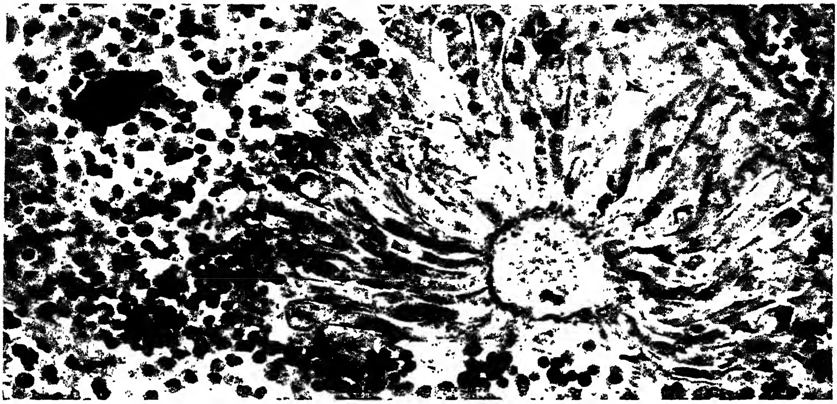


Fig. 155.—Pulmonary aspergillosis in man showing a fruiting heat and spores in a cavity in the lung tissue. The spores are slightly smaller than red cells and show a crenated edge when viewed from the end and a banded appearance from the side. Iron hematoxylin; × 650 (Humphreys).

lesions which may or may not be fatal. Henrici[61] has shown that *A. fumigatus* produces an endotoxin.

Pathogenicity for Man.—Human cases of aspergillosis are most frequently infections of the external ear (otomycosis). The disease varies from a plugging of the external meatus with a mass of mycelium to ulceration of the walls and even penetration of the middle ear; the mild cases are the most numerous. This type of aspergillosis is said to be relatively common in India, less so elsewhere though a considerable proportion (1 per cent) of ear infections in Germany have been reported to be aspergillosis. While *A. fumigatus* is the most common invader, *A. nidulans*, *A. flavus* and *A. niger* are sometimes found.

Infection of the lung is rare in man and probably is often secondary, particularly to tuberculosis. The disease is clinically similar to and may be mistaken for tuberculosis: there is extensive cavitation and it advances

[61] Henrici: Jour. Immunol., 1939, *36*:319.

rapidly. The mycelium may be found in the sputum and the fungus is readily isolated by culture but other etiologic agents must be excluded. Most of the reported cases of lung infection are among bird-fanciers, especially those having to do with the care of pigeons; there is convincing evidence that transmission of the disease from pigeons to man can take place.

Very rarely aspergillosis may take other forms in man. The occurrence of *Aspergillus* in maduromycosis has been noted earlier and a case of chronic suppuration with discharge of grains has been reported. It has been contended by some workers that fungi of the *A. nidulans* group may be responsible for certain types of splenomegaly but this appears doubtful.

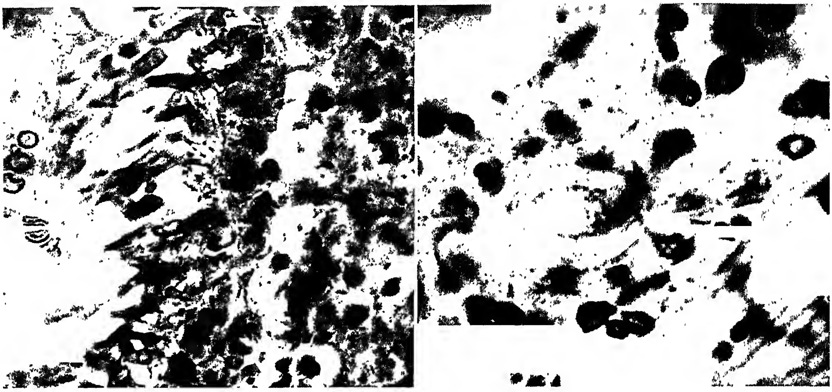


Fig. 156.—Primary mycotic bronchitis in man resulting from infection with *Aspergillus* sp. Left, mycelium invading the wall of the bronchus; × 950. Right, a segment of swollen mycelium in the deeper tissues under higher magnification; × 1550. Hematoxylin and eosin (Humphreys).

Fonsecaea Pedrosoi (Chromoblastomycosis)

Chromoblastomycosis was discovered by Pedroso in 1911 but his observations were not reported until 1920,[62] the first case in the literature being reported from Boston by Medlar in 1915.[63] The number of recorded cases is relatively small; something over one hundred authentic cases have been reported altogether and the sixth case in this country was reported in 1941.[64] The geographical distribution is wide, however, with the greatest number of cases found in Puerto Rico and Brazil while others have been observed in other parts of South America, Africa and the Far East. Chromoblastomycosis has been of increased interest in recent years and present knowledge of the disease has been summarized by Emmons[1] and by Carrion.[65]

[62] Pedroso and Gomes: Ann. Paulistas de Med. e Cir., 1920, *11*:53. Quoted by Emmons and Carrion.
[63] Medlar: Jour. Med. Res., 1915, *32*:507.
[64] Emmons, Hailey and Hailey: Jour. Amer. Med. Assn., 1941, *116*:25.
[65] Carrion: Mycologia, 1942, *34*:424.

The causative fungus is closely related to the *Hormodendrum-Cladosporum* group and was named *Hormodendrum pedrosoi* by Brumpt. Three types of sporulation are observed, the predominant type differing from one strain to another, and this has led to the splitting of strains into several genera with considerable confusion resulting. The new genus *Fonsecaea* was created by Negroni in 1936 and has gained some general acceptance and the organism is, therefore, also commonly known as *Fonsecaea pedrosoi*. Since the *Hormodendrum* type of sporulation is not an outstanding characteristic, Carrion[65] has proposed that the name *Fonsecaea pedrosoi* be accepted as the only species and that four varieties, *typicus*, *cladosporioides*, *phialophorica*, and *communis*, be recognized on the basis of predominant type of sporulation. Mycelium is not formed in the tissues and the parasitic phase of the fungus is a brown sclerotic cell which divides by septation.

The disease is an infectious granuloma of the skin and subcutaneous tissues, occurring usually, though not always, on the feet and legs. It ordinarily begins with a small warty growth on the foot and extends upward through the development of satellite lesions. It almost always remains localized, metastases are very rare, and there are no constitutional symptoms. The disease develops very slowly; usually the case is of ten to fifteen years' duration at the time of examination, and some are known to have persisted for as long as forty years. In advanced cases there is some elephantiasis of the affected limb and great numbers of lesions. These vary somewhat and are of four general types: hard, elevated, pigmented nodules; large, cauliflower-like, prominent tumors; moderately elevated, dull red, scaly patches; and discrete or diffuse verrucous hyperkeratotic growths. The lesions are readily traumatized and the disease may be complicated by secondary bacterial infection and ulceration. The sclerotic cells appear as spherical bodies, perhaps 12 μ in diameter, with a thick membrane and granular protoplasm and often show internal septation. They may be observed in biopsy specimens either within giant cells or free in the tissues, and are demonstrable in the epithelial debris obtained by scraping the lesions. The fungus may be cultured from such scrapings or from infected tissue.

There appears to be no evidence of spread of the infection from one person to another. Since the disease tends to occur on the legs and feet of barefoot, outdoor laborers in the tropics, it seems probable that the fungus is a saprophyte normally present in soil or decomposing organic matter which, when introduced into the skin by a splinter or thorn or through some minor abrasion, may at times assume pathogenicity.

SPOROTRICHUM SCHENCKI (SPOROTRICHOSIS)

Sporotrichosis was first recognized by Schenck in this country in 1898[66] and a few years later in France by Beurmann and Ramond.[67] It has since been found all over the world, though the majority of reported cases are from the United States, especially from the Mississippi and Missouri

[66] Schenck: Bull. Johns Hopkins Hosp., 1898, *9*:286.
[67] Beurmann and Ramond: Ann. de Derma et Syph., 1903, *4*:678.

Valleys, and from France. The disease, together with blastomycosis, is perhaps the most common and most important of the serious mycoses.[68]

Fungi of the genus *Sporotrichum* are characterized by the production of pear-shaped conidia directly from the mycelium which arise both laterally and at the tips of hyphae. The characteristic arrangement is not apparent in smears from cultures and the spores are usually found free, but is readily demonstrable in slide cultures. The hyphae are considerably more slender, 2 μ in diameter, than those of most molds, The character of the growth on agar is different from that of other molds; the colony is first soft and creamy in consistency, becoming more firm as the culture grows older, and there is no cottony mass of aerial mycelium. The growth also becomes darker with age, being at first a light tan which deepens to a dark brown or even almost black. Mycelium is not formed in the tissues and the parasitic phase of the fungus is a cigar-shaped body resembling an

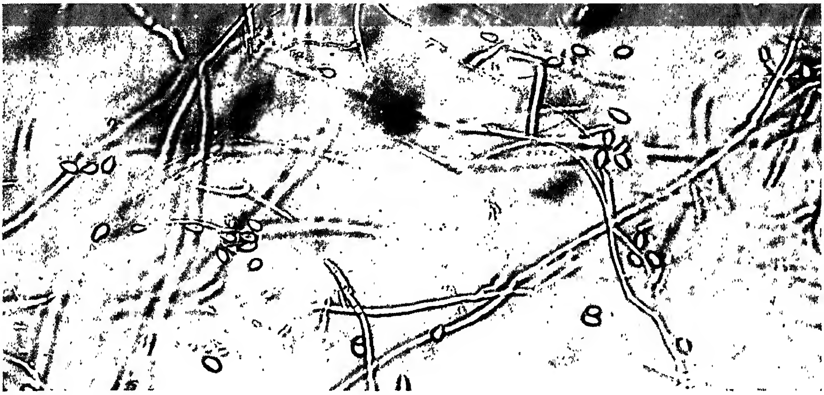


Fig. 157.—*Sporotrichum schencki* in culture. Spores and hyphae. Unstained preparation; $\times$ 450 (Rothman).

elongated yeast cell, 1 to 3 μ in breadth and 2 to 10 μ in length. These bodies are usually found within the leucocytes and apparently reproduce by fission. They are not spores.

The organism described by Schenck was named *Sporotrichum schencki* by Hektoen and Perkins.[69] That isolated in France was supposed to be a different species and came to be known as *Sporotrichum beurmanni*. The differences between the two are pigmentation, *S. schencki* being the lighter, the formation of fewer lateral spores by *S. schencki*, and the fermentation of sucrose but not lactose by *S. beurmanni* and the reverse by *S. schencki*. There also appear to be some differences in the clinical type of disease produced. These differences are not constant, however, and are subject to environmental modification, and it is apparent that the two are but a

[68] For general discussion see Beurmann and Gougerot: *Les Sporotrichoses*. Felix Alcan. Paris. 1912; Foerster: Amer. Jour. Med. Sci., 1924, *167*:54; *ibid.*, Jour. Amer. Med. Assn., 1927, *87*:1605.

[69] Hektoen and Perkins: Jour. Exp. Med., 1900, *5*:77.

single species, *S. schencki*. The name *S. beurmanni* continues to persist in the literature, however, giving the impression of two recognized species. The fungus infecting horses, *Sporotrichum equi*, is also identical with *S. schencki*.

Pathogenicity for Man.—The most common form of sporotrichosis in this country is cutaneous; the primary lesion, appearing at the site of some minor injury, often on a finger, fails to heal, ulcerates and is followed by the appearance of a series of subcutaneous abscesses along the course of the regional lymphatics. The subcutaneous lymph vessels can often be traced as reddened lines. Infection seldom extends beyond the regional lymph nodes and cases of generalized hematogenous infection are rare in this country though apparently more common in France. Metastatic lesions may appear in the liver or lungs and are most common in

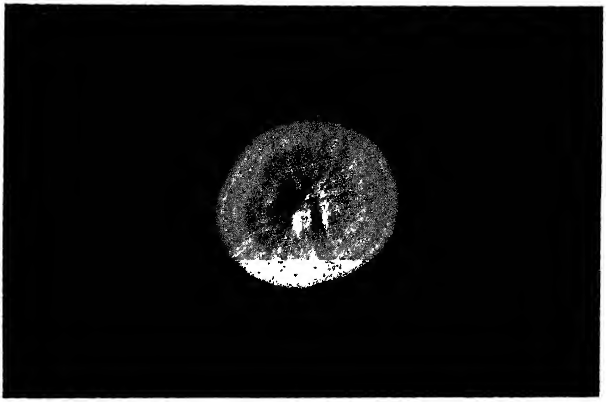


Fig. 158.—Giant colony of *Sporotrichum schencki* on Sabouraud's agar, three weeks old. × 2.

the testicles. The firm nodules in the skin are suggestive of syphilitic gummata and probably some cases of sporotrichosis have been so diagnosed. Henrici[1] has pointed out the common use of iodides in the treatment of tertiary syphilis in this connection and suggests that some cases of mistaken diagnosis may be apparently confirmed because of the therapeutic efficacy of these compounds in sporotrichosis.

Epidemiology.—Direct transmission of the infection from man to man has been observed but is very rare. A certain number of human infections have also been contracted, either directly through bites or indirectly by contact, from naturally infected horses and rats. In the great majority of cases, however, the fungus is introduced into the tissues from plants through some abrasion. Thus in fourteen of eighteen cases reported by Foerster[68] infection followed wounds by barberry thorns. The disease has been observed in florists and the fungus isolated from sphagnum moss.[70] It has been observed growing free on grains and Benham and Kes-

[70] Gastineau, Spolyar and Haynes: Jour. Amer. Med. Assn., 1941, *117*:1074.

ten[71] demonstrated its ability to grow on barberry and carnations; in the latter a bud rot was produced, an interesting example of infection of hosts as diverse as plants and man. It seems highly probable, therefore, that the fungus lives a saprophytic existence in nature, occasionally setting up an infection in man when mechanically introduced into the tissues.

Diagnosis.—The diagnosis of sporotrichosis is established by demonstration of the fungus. The cigar-shaped parasitic cell is gram-positive and may be found in gram-stained pus smears. These forms are relatively infrequent in human material, however, and sporotrichosis is not excluded by failure to find them. The fungus is readily cultivated on Sabouraud's medium from pus aspirated from unopened lesions. Rats are highly susceptible to infection and rat inoculation is of considerable diagnostic value. Male white rats are inoculated intraperitoneally; a pronounced orchitis occurs and there is a generalized peritonitis with minute nodules on all the peritoneal surfaces. The cigar-shaped cells are present in abundance in the rat lesions and may be found readily in gram-stained smears.

Immunity.—There is an immune response to infection manifested by the appearance of agglutinins (for spores) and complement-fixing antibodies. These are of some diagnostic value though somewhat non-specific in that sera from persons with thrush or actinomycosis will give positive reactions. A cutaneous test with *sporotrichin*, a preparation analogous to tuberculin, is of similar specificity.

The Dermatophytes[72]

By far the most common type of fungous disease of man is dermatophytosis (dermatomycosis), a superficial infection of the keratinized epidermis and keratinized epidermal appendages, the hair, hairsheaths and nails, the severity of which is dependent for the most part upon the location of the lesion and the species of fungus involved. Though certain other fungi, notably *Monilia*, produce clinically similar disease, a more or less homogeneous group of fungi, the dermatophytes, is responsible for the great majority of cases. The ability of these microorganisms to invade and parasitize the cornified tissues is closely associated with, and dependent upon, their common physiological characteristic, the utilization of the highly insoluble sclero-protein keratin.[73] The utilization of keratin is biologically rare and is shared by the dermatophytes only with a few species of saprophytic fungi and certain insects including the clothes moth (*Tinea*), the carpet beetles (*Dermestes*) and the biting lice (*Mallophaga*).

Though the various species of dermatophytes produce infections that are clinically characteristic, on the one hand single species may produce different types of disease, and on the other, infections that are very similar or essentially identical may be produced by different species, Further-

[71] Benham and Kesten: Jour. Inf. Dis., 1932, *50*:437.

[72] These organisms have been reviewed by Tate: Biol. Rev., 1929, *4*:41, and by Gregory: Biol. Rev., 1935, *10*:208. Later papers are discussed by Emmons: Bot. Rev., 1940, *6*:474.

[73] For studies on the metabolism of these fungi *cf.* Goddard: Jour. Inf. Dis., 1934, *54*:149.

THE COMMON DERMATOPHYTES

			Species	Disease in man	Geographic Distribution
Invading the hair and hair follicles	Small spore varieties		*Microsporum audouini*	Prepuberal ringworm of the scalp; suppuration rare	Commonest in Europe, producing about 90% of infections; about half infections in U. S
			Microsporum lanosum	Prepuberal ringworm of scalp and glabrous skin; suppuration not infrequent; kerion occasional	Uncommon in Europe; responsible for about half the infections in U. S.
			Microsporum gypseum	Prepuberal ringworm of the scalp and glabrous skin; suppuration and kerion common	Relatively rare in U. S.; common in South America
	Large spore varieties	Endothrix type	*Trichophyton acuminatum*	Ringworm of the scalp and smooth skin; onychomycosis; commonest in children but persists into adult life	Common in Europe; relatively rare in U. S.
			Trichophyton crateriforme	Ringworm of the scalp and smooth skin; onychomycosis	Common in Europe; uncommon in U. S.
			Trichophyton violaceum	Ringworm of the scalp and smooth skin; sycosis; onychomycosis; suppuration common, the hair follicles are atrophied	Common in Russia, Poland, Italy, Near East, but uncommon in U. S.
			Achorion schoenleini	Favus, in both scalp and smooth skin; onychomycosis; suppuration is the rule and kerion frequent	Common in Europe and the Far East; rare in U. S.
		Neo-endothrix type	*Trichophyton cerebriforme*	Usually ringworm of the glabrous skin and beard; ringworm of the scalp less frequent, onychomycosis	Common in Europe; uncommon in U. S.
		Ectothrix type	*Trichophyton mentagrophytes*	Commonest cause of intertriginous dermatophytosis of the foot ("athlete's foot"); ringworm of the smooth skin, suppurative folliculitis in scalp and beard	Ubiquitous
			Trichophyton rosaceum	Sycosis is the most common lesion; infection of smooth skin and nails	Common in Europe; sporadic distribution in U. S.
Not invading the hair and hair follicles			*Epidermophyton floccosum*	Cause of classic eczema marginatum of crural region; causes minority of cases of intertriginous dermatophytosis of foot; not known to infect hair and hair follicles	Ubiquitous, but more common in tropics
			Epidermophyton purpureum	Psoriasis-like lesions of smooth skin; onychomycosis, mild suppurative folliculitis in beard	Common in Far East, tropics and southern U. S.
			Endodermophyton concentricum	Commonest cause of tinea imbricata; infection of hair and nails uncertain	Common in South Pacific islands, Far East, India, Ceylon; reported in South America; does not occur in U. S.
			Malassezia furfur	Cause of pityriasis versicolor	Ubiquitous

more, other conditions such as chemical dermatitis, neuro-dermatitis and certain types of allergy may closely simulate dermatophytosis, but do not, of course, respond to treatment with fungicides. Demonstration of

the causative fungus by direct microscopic examination of pathological material or by isolation and culture is, therefore, highly desirable, especially in all cases presented for treatment.

The dermatophytes are mold-like fungi grouped with the Fungi Imperfecti; ascospore formation has been reported by some workers but has not been unequivocally established. These organisms differ from most other pathogenic fungi in that the cells are multinucleate, usually containing 4 to 6 nuclei, and division is amitotic. Arthrospores, chlamydospores and the individual cells of fuseaux are also multi-nucleate, but aleuriospores contain but a single nucleus.[74] The group is a homogeneous one, immunologically as well as morphologically and physiologically, and the fungi comprising it are more closely related to one another than to other fungi. The

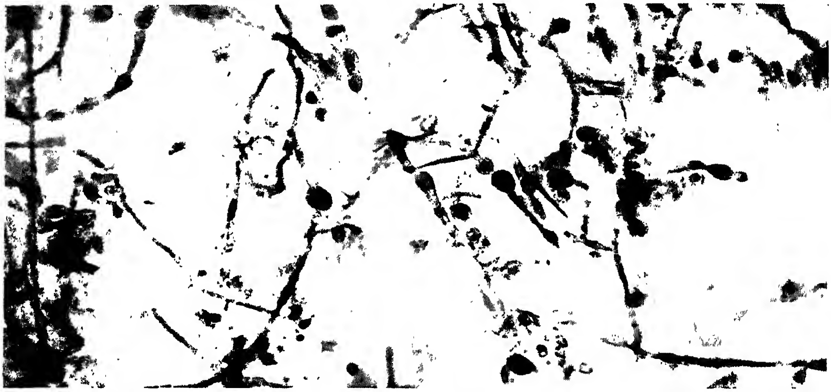


Fig. 159.—Chlamydospores, *Microsporum audouini.* Mounted in Annam's lactophenol cotton blue solution; × 420.

affinities of the group to the other fungi are not clear, however, and the dermatophytes have never been satisfactorily oriented in a general system of fungus classification; they constitute a group somewhat apart and are usually neglected by botanists.

Differentiation of Genera and Species.—The differentiation of species within the group is somewhat uncertain in a general sense. The classification of Sabouraud, presented in 1910[75] and somewhat modified by him later,[76] is by far the most generally used. It is based on both the clinical character of the infection and on colonial morphology, including pigmentation, the morphology of the reproductive and vegetative structures being a secondary matter. The species so differentiated are sound natural groups in spite of objections raised by some systematists and the classification and differentiation is practical and workable.

It is to be noted, however, that colonial morphology is readily altered

[74] *Cf.* Grigoraki: Ann. Sci. Nat. Bot., 1925, Ser. 10, *7*:165.
[75] Sabouraud: *Les Teignes.* Masson et Cie. Paris. 1910.
[76] Sabouraud: Ann. Derm. Syph., 1929, Ser. 6[e], *10*:236.

by continued culture on artificial media. In consequence many stock cultures of dermatophytes are atypical to a greater or lesser extent. Such variation is especially prone to occur on maltose "proof" agar and Sabouraud recommends a *milieu de conservation*, the same medium but lacking sugar, which does not bring out the differential characters of all species; growth on it is much slower, but it has the virtue of indefinitely postponing pleomorphic changes. Differential characteristics reappear, of course, on transfer to the maltose *milieu d'épreuve*.

Sabouraud described 45 species of dermatophytes in 1910 and new species have been described more or less continuously since. According to Gregory[72] at least 184 species have been reported as pathogenic for man by 1935, and Emmons[72] states that there are at least 200 species named in

Fig. 160.—Blunt end fuseaux, *Epidermophyton floccosum*. Note the attachment to the mycelium. Mounted in Annam's lactophenol cotton blue solution; × 420.

the literature. In many instances the differences on which these have been based are minor and it is probable that an appreciable proportion of these new species are no more than varieties and have little practical significance. Here we shall consider only the more important which account for the vast majority of the dermatophytoses.

Differential Characteristics.—It will be apparent that the primary differentiation of these organisms is made on a clinical basis, *i.e.*, division into the group of fungi which invade the hair and hair follicles, and the group of those which do not. While parasitic on the skin and its appendages, the thallus is differentiated only into hyphae and arthrospores; the latter, arising by fragmentation of the mycelium, are to be regarded as oidia as indicated earlier though usually termed "spores" by dermatologists. The variety of differentiated structures, including aleuriospores, spindle-spores or fuseaux, and vegetative structures such as spirals, pectinate bodies, nodular organs and raquet mycelium, appear on cultivation on artificial media.

In the first group there are, for present purposes, two genera which dif-

fer with respect to size and arrangement of the spores formed in the tissues. The genus *Microsporum* includes the small spore type (3–4 μ in diameter), and the genus *Trichophyton*, the large-spore type (7–8 μ in diameter). This distinction is not absolute, however, for the very common species, *Trichophyton mentagrophytes*, forms small spores. Furthermore, the spores differ in arrangement. While the mycelium of *Microsporum* grows within the hairs, spores are formed only outside of the hair and occur in irregular clusters in a kind of mosaic arrangement. The spores of *Trichophyton*, on the other hand, occur in chains inside or outside the hair.

Further differentiation of *Trichophyton* is made on the basis of location of the growth with respect to the hair. The *endothrix type* (sometimes called *Endotrichophyton*) grows within the hair, and mycelium and chains of spores are found there. The *neo-endothrix type* grows for the most part within the hair but in some hairs growth occurs on the surface. Finally,

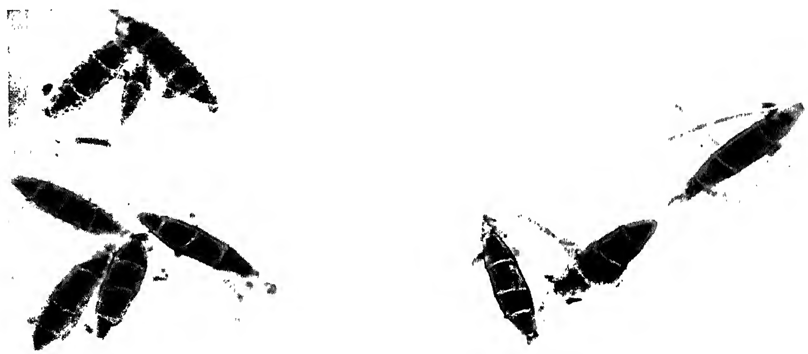


Fig. 161.—Pointed fuseaux, *Microsporum gypseum*. Mounted in Annam's lactophenol cotton blue solution; × 420.

the *ectothrix type* (sometimes called *Ectotrichophyton*) grows only on the surface and the hyphae and chains of spores are found there. The genus *Achorion* is distinguished from *Trichophyton* by the production of yellow crusts or scutula (see below) on the scalp. It is an endothrix type and many workers believe that it should be included in the genus *Trichophyton*.

It will be clear that differentiation of these genera can be closely approached by, first, the clinical character of the disease and, second, by direct microscopic examination of epilated infected hairs; but identification is possible only by culture. In general *Trichophyton* infections show a characteristic tendency to produce an inflammatory reaction with deep infiltration of the skin that is rarely produced by *Microsporum* infections; this difference is of some value in distinguishing between *Microsporum* infections and infections with small-spore ectothrix *Trichophyton*. The animal strains of *Microsporum* such as *M. lanosum*, however, also elicit inflammatory reactions.

Three genera of dermatophytes which do not invade the hair are considered here. Species of *Epidermophyton* invade the superficial layers of the skin and the fungi are found in scales of the epidermis taken from the periphery of the lesion as articulated filaments of mycelium breaking up into chains of round to oval arthrospores. *Endodermophyton* characteristically grows within the epithelium between the superficial and deeper layers but does not invade the corium; these forms are very difficult to cultivate. *Malassezia* is somewhat apart from the other dermatophytes and is not well known (see below) though the clinical disease is characteristic.

Pathogenicity.—At present the dermatophytes are known only as parasites and are usually assumed to be obligate parasites either of man alone or of man and animals. The majority grow readily on laboratory media and also grow on such substrates as cereal grains, shed hair, horn

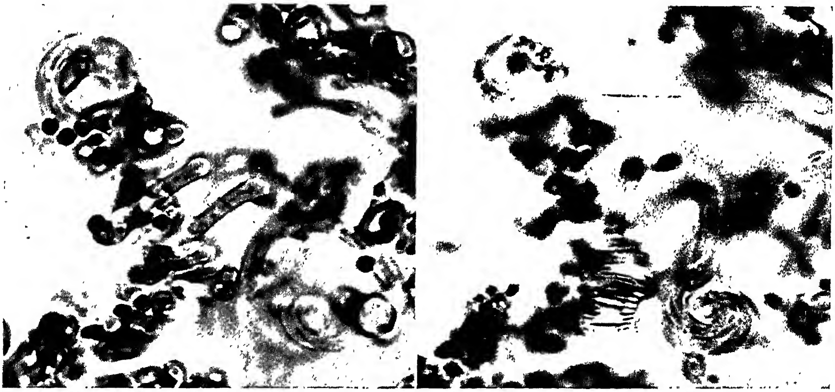


Fig. 162.—Spiral hyphae, *Trichophyton mentagrophytes* var. *granulosum*. The same spiral appears in different planes of focus. The small dark bodies are microconidia. Mounted in Annam's lactophenol cotton blue solution; × 950.

debris, sterilized fragments of straw in moist tubes, etc. and will remain viable in litter containing such materials for two to three years. If protected from dryness they may live on the wooden floors of shower rooms, dressing cabins, mats, etc. for a considerable period of time. It has, therefore, been urged by some that the dermatophytes may live a saprophytic existence in nature, unrecognized because of the regular formation of a variety of differentiated structures. In any case, a saprophytic stage in the transmission from one animal to another seems possible—Davidson and Gregory[77] have suggested the shedding of infected hairs and scales—and is suggested by the findings of Muende and Webb[78] who cultured two species of *Trichophyton* from dung in a shed occupied by infected calves.

The dermatophytoses show, in many instances, a pronounced age and

[77] Davidson and Gregory: Nature: 1933, *131*:836; Canadian Jour. Res., 1934, *10*:373.
[78] Muende and Webb: Arch. Derm. Syph., 1937, *36*:987.

sex distribution and there is some difference in the geographical distribution of the various species. Common ringworm or "gray patch" of the scalp is confined to the young, occurring more often in boys than in girls, and is rare after puberty. Others, such as *Epidermophyton* species, occur for the most part in adult males. The distribution of intertriginous dermatophytosis of the feet, commonly known as "athlete's foot," in the young adult male is probably in large part an expression of risk. *Microsporum lanosum* is more common in this country than in Europe and the reverse is true of *Achorion schoenleini*, while *Endodermophyton concentricum* is well known in the tropics and certain parts of the Far East but rare in temperate climates.

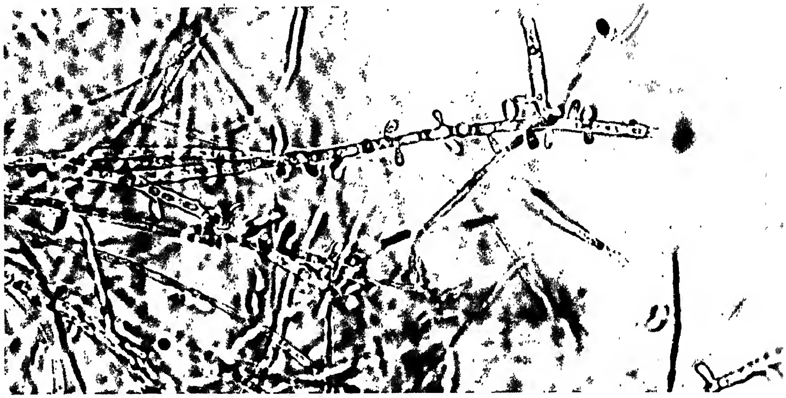


Fig. 163.—*Hyphae sporiferae* (*thyrsi sporiferae, thyrsi*) in culture of *Trichophyton purpureum*. The attachment of aleuriospores, directly or by very short stalks, to the hyphae is evident. Unstained preparation; × 450 (Rothman).

Some dermatophyte species appear to be so closely adapted to man that they are unable to infect lower animals while others not only produce infections in experimental animals, but animals such as the cat and dog are natural hosts and human infection may be acquired from them. There is also a high degree of specificity as to the tissues attacked. While, as indicated earlier, these fungi are well adapted to parasitize the horny layer of the epidermis, they appear to be unable to invade and infect other organs of the body. The intravenous injection of *Achorion* or *Microsporum* spores or emulsions of virulent *Trichophyton mentagrophytes* does not produce an infection of the internal organs of susceptible animals; rather, the microorganisms introduced tend to become localized in the skin and to develop where it is damaged as by scarification.

Growth of the fungus in the skin and hair is more or less equal in all directions and the lesions produced tend to have a circular form. For this reason the Greeks named the disease *herpes*, a term which still persists though modified as *herpes tonsurans*, *herpes circinatus*, or *herpes desquamans* to distinguish the dermatophytoses from herpetic infection of virus

etiology, herpes simplex in its various manifestations, and herpes zoster of as yet unknown etiology (*cf.* p. 855). The Romans associated the lesions with lice and named the condition *tinea*, meaning any small insect larva. This name is likewise in common use. The English *ringworm* is, of course, a combination of the Greek and Roman terms.

With any species of dermatophyte, infection begins in the horny layer of the epidermis. Those which infect the hair follicles, hair and nails soon invade these structures, often producing little more than a scaling of the epidermis. Those which remain in the epidermis affect the drier parts of the skin including the palmar and plantar surfaces, or the moister regions in the inguinocrural fold and the interdigital spaces. Thus a wide variety

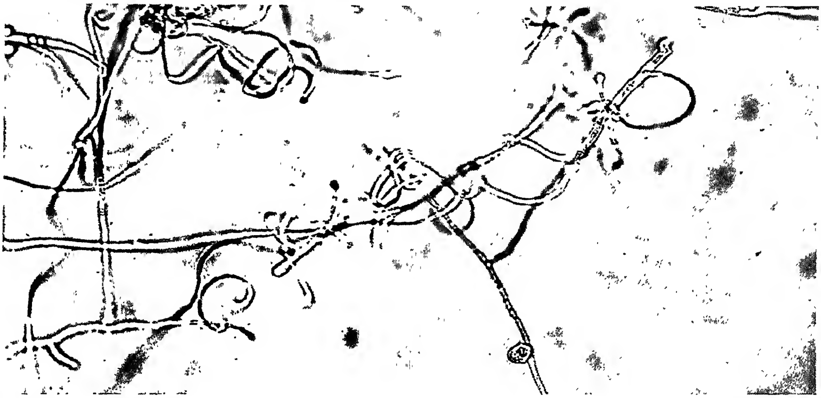


Fig. 164.—Spiral hyphae in culture of *Trichophyton mentagrophytes* var. *gypseum*. Unstained preparation; × 450 (Rothman).

of clinical types may be observed, but the differences are more apparent than real for the pathologic changes are fundamentally the same in all types.

Infection of the Dry Glabrous Skin.—The formation of characteristic concentric rings of inflammatory reaction is more evident in infections of the smooth skin than in hirsute areas. Two types of lesion are produced, the one dry and scaly, and the other vesicular. The first begins as a small elevated area of inflammation which spreads, the margin remaining red and sometimes slightly swollen while the central area becomes covered with small scales; spontaneous healing occurs in the center as the circinate margin advances.

In the second type vesicles appear irregularly or immediately back of the advancing hyperemic and elevated margin; a crust is formed, followed by spontaneous healing in the center to leave a more or less pigmented area. The lesion may become pustular with invasion of the hair follicles. In either case the fungi are most numerous at the margin of the advancing lesion, and scales or vesicles from this region are best suited for study and isolation of the microorganism.

Infection of the palmar and plantar surfaces (the latter is the more common) is ordinarily secondary to infection of the interdigital spaces. Vesicles are formed with the thick horny layer of the sole separated from the epidermis beneath; the vesicles may be filled with fluid but soon dry. When the top peels off it leaves a pink area surrounded

by a border of loosened and peeling skin. The formation of secondary vesicles and continuous repetition of the process produces widening areas of pink or reddish skin, the dysidrotic form of the disease. The formation of fissures, common in the interdigital areas, may also occur on the sole of the foot. A dry, hyperkeratotic type of lesion without vesiculation is produced in the chronic infections.

Infection of the Epidermis of the Principal Folds.—In these regions there are also two main types of dermatophytosis, the one, *eczema marginatum*, chiefly confined to the crural fold, and the dysidrosiform intertriginous mycoses of the hands and feet. In the former a small round swollen area of inflammation appears which spreads, producing at first a circinate lesion which later becomes irregular owing to differences in rate of spread. The actively developing margin is an inflamed area in which there are numerous small vesicles filled with a serous exudate. In most instances the infection begins on the thigh where it is in contact with the scrotum, and spreads rapidly, usually involving the inner thigh and sometimes the gluteal and pubic regions. Pruritus is a common symptom.

In the interdigital areas, usually of the toes (moniliasis is the more common in the drier areas between the fingers) the epidermis is macerated or it shows circinate scaling. Small eroded patches are common. Vesicles are rarely seen because they rupture early,

Fig. 165.—Chlamydospore (upper left) and raquet mycelium (lower right) in culture of *Microsporum audouini*. Unstained preparation, oblique lighting; × 950 (Rothman).

leaving circular or polycyclic epithelial fringes. Deep within the fold the epidermis is membranous and swollen, fissures form and the macerated epithelium is shed as moist, white scales.

Infection of the Hair and Hair Follicles.—Invasion of the hair follicles and hair produces two general types of lesions. The most distinct and first to be differentiated clinically is that of *favus*, produced by *Achorion schoenleini* and distinguished by the formation of the *favic scutulum*. Beginning with a small spot or scaly inflammation, crusts appear as yellow points about the hair follicles. The hyphae and spores form a ring about the follicular orifice and a leucocytic infiltration produces an intraepidermic pustule. A yellow disk appears at the base of the pustule which enlarges with the lesion to produce the cup-shaped yellow crust known as the scutulum, which in time often becomes as large or larger than a pea. On the upper or concave surface the epidermis is intact and the hair is brittle, lusterless and gray, but not necessarily broken, while the epidermis below is infiltrated and sclerosed. Exfoliation of the horny layer occurs, some of the upper layer of the scutulum being removed. The scutulum itself consists of a mass of fungus mycelium in an enlarged hair follicle; the center surrounding the hair is a tangle of broken hyphae and spores inside an area of regular hyphae in the epidermis, and at the periphery the hyphae are perpendicular to the surface. Infection of the hair begins at

the root and some time, usually three to four months, is required for the fungus to grow up through the hair to produce the dull, powdery gray, dry appearance characteristic of favus. The hair remains, though seldom more than a few centimeters in length, for several years before being shed through scar formation in the follicle, but in time it loosens, falls out, and may not return, resulting in patches of permanent alopecia. Section of infected hairs shows air spaces, presumably left by dead hyphae; relatively few hyphae remain and the hair structure is clearly visible.

The second type of infection includes ringworm of the scalp or *tinea tonsurans*, which is much the more common in this country, and infection of the beard. It may be subdivided into *Microsporum* infection, endothrix *Trichophyton* infection, and suppurative infections usually with ectothrix *Trichophyton*.

In *Microsporum* ringworm the fungus invades the hair follicle to form a mass of mycelium between the hair and the epithelium. Some hyphae penetrate the hair and grow downward, while others grow on its surface to form the characteristic layer of spores. The lesion begins as a small erythematous area. Within a few days it pales and becomes scaly and the hair assumes the characteristic grayish, discolored appearance, becomes weakened and breaks off a few millimeters above the scalp. The lesion spreads in the

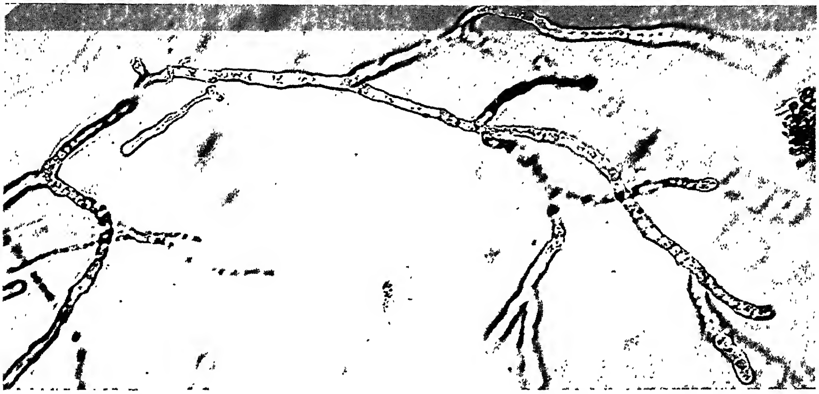


Fig. 166.—Branched hyphae or favic chandelier of *Achorion schoenleini*. Unstained preparation; × 600 (Rothman).

characteristic ring form and may coalesce with other infected areas produced by autoinoculation to give an irregular area of infection in which all the hairs are affected. There is little inflammatory reaction.

Trichophyton ringworm differs from the *Microsporum* infection in a number of respects. While infection of the hair follicle and hair follows essentially the same course, the lesions are small with often only two or three affected hairs and all the hairs within a given lesion are not affected, and the lesions are multiple, numerous and scattered over the scalp. The infected hairs break off sharply at the follicular orifice in *Trichophyton acuminatum* infections, and somewhat above to leave stumps in *Trichophyton crateriforme* infections. There is somewhat more inflammatory reaction than in microsporiasis, and often spread of the infection to the glabrous skin.

Infections with ectothrix *Trichophyton* are distinguished clinically by a more marked inflammatory reaction and often suppurative folliculitis. There is an infiltration of serum and leucocytes into the skin of the affected area and an infiltration of the deeper tissues. In areas of contiguous suppurative folliculitis an indolent cutaneous-subcutaneous infiltration called *kerion* is formed which is boggy on palpation, pus oozing from the follicles on pressure. Suppurative lesions of the beard are termed *sycosis*. Suppurative infection and kerion may, of course, occur with other species, including endothrix *Trichophyton* and *Microsporum* of animal origin.

It was observed by Margarot and Devéze[79] in 1925 that infected hairs and fungus cultures show fluorescence in ultraviolet light.[80] This empirical observation has proved to be of very considerable practical value, especially in *Microsporum* ringworm of the scalp. It is generally agreed that all hairs infected with *Microsporum* show a brilliant greenish fluorescence and those infected with *Achorion schoenleini* a greenish but less brilliant fluorescence, both distinct from the bluish tint of normal skin. There appears to be some disagreement with respect to *Trichophyton* infection; it is reported by Davidson and Gregory[81] that in infection with some species the hairs fluoresce but in others they do not, while Lewis and Hopper[1] state that all endothrix *Trichophyton* species show a dull bluish fluorescence of the infected hair though hair infected with ectothrix *Trichophyton* does not fluoresce. Davidson and Gregory[81] have found that the fluorescence is due to the presence of a substance in the hair which can be extracted either with warm water after a preliminary ether extraction, or with dilute alkali.

Infection of the Nails.—Infection of the nails, *tinea unguium* or *onychomycosis*, may be either primary or secondary to an epidermal lesion. The infected nails are usually opaque, lusterless, yellowish and friable and varying degrees of dystrophy are found. The infection ordinarily begins under the free border or along the lateral margin of the nail, producing an opaque, yellowish-white irregular spot beneath the central area. This may develop into a thick spongy friable layer, readily detachable. As the nail plate separates from the bed yellowish or white longitudinal streaks usually appear, it becomes thin and brittle and breaks off; sometimes the entire nail plate is lost. When the surface of the nail is attacked early, it becomes fissured and soft and wears away. In general, there is no marked inflammatory reaction. The disease is of long duration, and may last ten years or more, and there is little if any tendency to spontaneous healing. The disease is usually due to *Trichophyton* but *Microsporum* and *Epidermophyton* infections have been observed.

Immunity.—Though an acquired immunity to infection has been demonstrated in experimental animals by a number of workers, the status of an effective immunity in man is uncertain. There have been some enthusiastic reports of the efficacy of vaccine therapy which are, perhaps, open to question. Much of the work that centers about local immunity appears to be uncritical, *viz.*, in many cases remarkable results have been reported but have not been regarded by immunologists generally as contributing materially to a solution of the general problem of local immunity (p. 294). Hypersensitivity, however, is a common, though not invariable, manifestation of the immune response to dermatophyte infection, and desensitization procedures appear to have definite therapeutic value in certain cases.

Hypersensitivity is manifested in two ways. One of these is the appearance of secondary, non-parasitic lesions on parts of the body remote from the infection. These are termed "ids," in a general sense *mycid* or *dermatophytid*, and more specifically *microsporid*, *trichophytid* and *epidermophytid*. The mycid takes the form of a symmetrical eruption over relatively large areas, usually of the trunk, as a rash. The eruption may be vesicular with sterile content, papular, or lichenoid, and is sometimes localized at the follicular pores. A rather frequent occurrence is a sterile vesicular eruption on the hands secondary to infection of the feet. It is generally

[79] *Cf.* Margarot and Devéze: Ann. Derm. Syph., 1929, Ser. 6ᵉ, *10*:581.

[80] "Black light," commonly known as Wood's light because the radiation is filtered through Wood's nickel oxide glass which holds back almost all the visible rays but passes the longer ultraviolet rays.

[81] Davidson and Gregory: Canadian Jour. Res., 1932, *7*:378.

bel eved that spores or bits of mycelium from the infection lesion enter the blood stream and are eventually deposited in the skin where they induce the local allergic response with destruction of the fungus elements. This view is based in part on a number of successful isolations of these fungi from the blood stream during the development of mycids. It is not known what part, if any, soluble substances liberated by the dissolution of the fungi play in the phenomenon.

ASSOCIATION OF DERMATOPHYTE SPECIES WITH CLINICAL CHARACTER OF THE INFECTION

(1) Lesions of the smooth skin:
- (a) Lesions of the moist epidermis of the principal folds:
  - (i) Eczema marginatum, usually confined to the crural region:
    EPIDERMOPHYTON
  - (ii) Dysidrosiform intertriginous mycoses of the feet and sometimes hands:
    EPIDERMOPHYTON
    ectothrix TRICHOPHYTON
- (b) Circinate lesions on the backs of the hands, arms, face, neck and trunk:
  - (i) Intricate pattern of concentric rings—tinea imbricata:
    ENDODERMOPHYTON
  - (ii) Reddish patches, not raised, with actively advancing margin and a tendency to central healing:
    usually MICROSPORUM
    sometimes endothrix TRICHOPHYTON
  - (iii) Reddish, elevated, scaly plaques, usually pustular at the margin:
    ectothrix TRICHOPHYTON

(2) Lesions of the hair and hair follicle:
- (a) Scutula present. hairs tend to split longitudinally and not break off transversely:
  ACHORION
- (b) No scutula present, hairs tend to break off transversely:
  - (i) No suppuration, as follicular abscess, pustules, kerion:
    1. Hairs broken off at uniform height several millimeters above the skin, scaling, spores in irregular clusters on the outside of hairs:
       MICROSPORUM
    2. Hairs usually broken off flush with the follicular orifice, little scaling, mycelium and chains of spores within the hair:
       endothrix and neo-endothrix TRICHOPHYTON
  - (ii) Suppuration, lesions of smooth skin often present, fungus both in and on the hair, spores largely external in chains:
    ectothrix TRICHOPHYTON

(3) Lesions of the nails, no distinctive clinical features:
ectothrix TRICHOPHYTON
EPIDERMOPHYTON
ACHORION

Hypersensitivity may also be demonstrated by the injection or application of preparations of dermatophyte cultures analogous to tuberculin and called *trichophytin*. The local and constitutional reactions that may be produced with trichophytin are much the same as those to tuberculin. The test does not differentiate between *Microsporum* and *Trichophyton* or their species for all appear to contain a common or very closely related antigen. The utility and reliability of the trichophytin test in diagnosis is a subject of conflicting opinion. It is maintained by some, such as Wise

and Wolf[82] and Lewis, MacKee and Hopper,[83] that the test is a valuable adjunct to other methods of diagnosis when used with the proper precautions; others, however, such as Swartz,[1] regard the test as of questionable value because, on the one hand, infection does not always result in sensitization, and, on the other, a positive reaction may only indicate a past infection unrelated to the condition in question.

Characteristics of Dermatophyte Species.—The characteristics of the more important species of dermatophytes may be considered briefly:

Microsporum audouini (*Sabouraudites audouini, Closteroaleuriosporia audouini*): This fungus is the classic etiologic agent of common prepuberal ringworm of the scalp; it is relatively more common in Europe, where it causes perhaps 90 per cent of the infections, than in this country where it is found in about half the cases. An inflammatory reaction is produced only occasionally, suppuration is uncommon, and kerion very rare. The trichophytin reaction is usually slight or absent.

Microscopic examination of the epilated hair stub shows hyphae within the hair and external irregular clusters of spores. The colony on maltose agar begins as a white fluffy mass which develops a central elevation and radial furrows and becomes grayish to buff in color as it matures. Aerial growth is scanty. About two weeks' incubation suffices for full development of colonial character. In slide culture fuseaux are rare and conidia few, but arthrospores, pectinate bodies and raquet mycelium are abundant. Experimental animals are highly resistant to infection.

Microsporum lanosum (*Microsporum canis, Microsporum caninum, Sabouraudites lanosus, Closterosporia lanosa*): *Microsporum felineum* is regarded by some as a separate species and by others as only a variety of *M. lanosum.* The fungus is the cause of half or more of the cases of ringworm in children in this country, but is found in only a small minority of cases in Europe. It differs from *M. audouini* in that it is a natural parasite of lower animals—it was originally found in the dog—and children may contract the disease from infected kittens and puppies as well as from one another. Infections of the smooth skin are common and produce a circinate lesion with a vesicular border and a mild erythema. In the scalp there is a mild inflammatory reaction and the infection is not infrequently suppurative; kerion occurs in 2 to 3 per cent of the cases. The trichophytin reaction is usually strongly positive.

In the epilated hair the fungus is indistinguishable from *M. audouini.* Colonial growth is somewhat more rapid and a yellowish pigment is produced, the mature colony being tan and the medium a yellowish color. Both radial and concentric striations or furrows are often present and there is an abundant and wooly aerial growth. Pleomorphism after four to five weeks' incubation is the rule. In slide culture fuseaux are abundant in the center of the growth; other structures such as conidia, raquet mycelium and pectinate bodies are not striking. Kittens, puppies, rabbits and guinea pigs are readily infected experimentally.

Microsporum gypseum (*Microsporum fulvum, Closterosporia fulva, Achorion gypseum*): This organism is present in South America and is apparently imported into this country where it is found only occasionally, Like *M. lanosum,* it is an animal parasite and is one cause of dog favus. The disease in the scalp is similar to that produced by *M. lanosum* but with more marked inflammatory reactions as a rule, and suppuration and kerion are common. The smooth skin may be infected. The trichophytin reaction is strongly positive.

In infected hairs the spores tend to a linear arrangement in the early stages of the disease, and later the picture is indistinguishable from that produced by *M. audouini* and *M. lanosum.* Colonial morphology is highly characteristic; a central umbo, which may be white, appears early and the colony is a cinnamon to pinkish brown. Concentric furrows may be present. In slide culture fuseaux are numerous, and raquet mycelium and nodular organs may be found together with moderate numbers of conidia. The rabbit, cat and guinea pig may be infected.

[82] Wise and Wolf: Arch. Dermat. Syph., 1936, *34:1.*

[83] Lewis, MacKee and Hopper: Arch. Dermat. Syph., 1938, *38:*713.

Trichophyton acuminatum (*Trichophyton sabouraudi, Aleuriospora acuminata*): This fungus is one of the common causes of trichophyton ringworm of the scalp in Europe. Like other trichophytoses, *T. acuminatum* infections, though more common in children, persist into adult life. Infection of the smooth skin occurs with the formation of a circinate lesion with vesicular border. Infection of the nail occurs. The trichophytin reaction is positive.

The fungus is of the endothrix type and chains of spores are found within infected hairs. The colony is acuminate, *i.e.*, there is a flattened conical peak projecting from its center, with radiating furrows and peripheral folds. It is creamy white in color and the surface is fine and powdery. In slide culture there is an abundance of round to oval micro conidia, borne laterally or on the tips of hyphae, as thyrses and *en grappe*. Chlamydospores are present but there is no characteristic predominance of vegetative structures. A mild localized infection may be produced in the guinea pig.

Trichophyton crateriforme (*Trichophyton tonsurans, Chlamydoaleuriospora crateriformis*): This is the other common ringworm trichophyton found in Europe (according to Sabouraud *T. acuminatum* and *T. crateriforme* cause 72 per cent of *Trichophyton* infections) but is not often encountered in this country. A mild inflammatory reaction occurs in the scalp, and lesions of the glabrous skin are crusted rather than scaling. The nails may be infected. The trichophytin reaction is negative or weakly positive.

The spores present in chains within infected hairs tend to be more cylindrical than round and have the appearance of a ladder rather than a string of beads. Growth is relatively slow. the colony is creamy in color and velvet-like in appearance, and is crateriform, *i.e.*, sunken in the center with a raised ring at the periphery. Morphology in slide culture is very similar to that of *T. acuminatum*. A very mild, spontaneously healing lesion may be produced by guinea pig inoculation.

Trichophyton violaceum (*Favotrichophyton violaceum, Bodinia violacea, Arthrosporia violacea*): This fungus is widely distributed over the world and is especially prevalent in Russia, Poland, Italy and the Near East. In this country it is usually, though not always, found in immigrants. The lesions on the scalp are much the same as those produced by other species of *Trichophyton;* the formation of small pustules and follicular crusts is common. Similar lesions are produced in the beard and the infection often spreads to the nails. The trichophytin reaction is negative or faintly positive.

In the infected hair the morphology of *T. violaceum* is that of typical endothrix *Trichophyton*. Colonies grow slowly and remain relatively small. The colony is of the acuminate type and in recently isolated cultures is a deep violet in color, but pigmentation is readily lost on continued cultivation. Conidia are not formed in culture and the surface remains smooth and without a powdery coat. No characteristic structures are found in slide cultures. Guinea pigs, dogs and cats have been infected by experimental inoculation.

Achorion schoenleini (*Trichophyton schoenleini, Arthrosporia schoenleini, Grubyella schoenleini*): Favus is prevalent in Eastern Europe and the Far East but relatively uncommon in this country. It is characterized by the formation of the favic scutula described earlier. The formation of scutula is the rule on the scalp and they may be found in infection of the skin, or the skin lesions may be vesicular or scaly. Infection of the nails with *A. schoenleini* produces severe dystrophy of the nail plates. Most human cases are infections with this species but related forms produce disease in animals, such as *Achorion quinckeanum* of mouse favus, and are occasionally transmitted to man.

Infected hairs show large spores in chains and coarse filaments within the shaft and, characteristic of this infection, air bubbles left by degeneration of the fungus elements within the hair. The distribution of the fungus in the scutulum has been described earlier. Growth in culture is slow and usually three weeks are required for development of the colonies. They are yellowish and waxy in appearance at first, and later usually become wrinkled and whitish with aerial mycelium. The fungus is highly pleomorphic in slide culture, showing various irregular bodies, swollen and distorted hyphae, etc., but the branched structure known as the favic chandelier is readily found and diagnostic for it is not formed by other species of dermatophytes. The fungus is pathogenic for mice, and nodular lesions of the lungs or peritoneum may be produced in the rabbit by intravenous or intraperitoneal inoculation respectively.

Trichophyton cerebriforme (*Trichophyton flavum, Neotrichophyton flavum*): This fungus infects the beard and smooth skin and, more rarely, the scalp. It is common in England, France, Germany and Italy, less so elsewhere. The lesions are indistinguishable from those produced by *T. crateriforme.*

The location of the fungus elements in the infected hair is, for diagnostic purposes, of the endothrix type and very similar to that of *T. crateriforme.* The young colony is white and crateriform but as it matures the central depression tends to fill out, the folds become prominent and it becomes yellowish in the center. The morphology in slide culture is very similar to that of *T. acuminatum* and *T. crateriforme.* Experimental infection may be produced in guinea pigs and the fungus has been reported to occur naturally in cats.

Trichophyton mentagrophytes (*Trichophyton gypseum, Trichophyton asteroides, Trichophyton pedis, Trichophyton interdigitale, Trichophyton niveum, Ectotrichophyton mentagrophytes, Sabouraudites asteroides, Aleuriospora rosacea, Ctenomyces mentagrophytes, Spiralia mentagrophytes*): This fungus is by far the commonest cause of dermatophytosis of the foot (athlete's foot), and also produces tinea circinata and follicular infection of the scalp with kerion as well as onychomycosis. The number of synonyms is due in part to the pleomorphism of the fungus; the first five given above have been commonly used in the past to denote different but closely related species. It is becoming increasingly apparent, however, that these forms lie in continuous series between the granular *T. gypseum* varieties and the floccose type of growth characteristic of *T. interdigitale.* Here we shall follow the suggestion of Emmons[72] and include them all in the single species *T. mentagrophytes.* It may be noted also that *Trichophyton granulosum* and *Trichophyton felineum* are so closely related to *T. mentagrophytes* that they can hardly be regarded as more than varieties of that species. Trichophytids associated with intertriginous infection are usually due to this fungus. The trichophytin reaction is positive.

Infection of the hair is of the ectothrix type and, as noted earlier, the fungus is a small-spore type. The hair is usually not broken and the external spores are relatively few in number and in linear arrangement in contrast to *Microsporum.* In scales and macerated tissue hyphae and spores are found but are not characteristic. There are four general types of growth with respect to colonial morphology. (1) The type most often found produces a white fluffy colony that becomes flat, velvety and yellowish-buff in color as it matures. Slide cultures are characterized by the presence of spirals. Nodular organs, raquet mycelium, pectinate bodies, chlamydospores and fuseaux may be found together with conidia in thyrsi and *en grappe.* (2) The white fluffy type with aerial mycelium is often called *T. interdigitale.* Fuseaux and spirals are usually absent in slide cultures; the aerial mycelium is largely vegetative with conidia in small clusters, and nodular organs and raquet mycelium may be found. (3) The granular, powdery, light tan to yellow colony is the *T. gypseum* type of growth. The surface is velvety and tends to become fluffy with age. Slide culture shows few spirals but numerous fuseaux and conidia *en grappe* and as thyrsi. (4) The white colony of the *T. niveum* type begins as fluffy but becomes compact and shows surface irregularities with maturity. The morphology of the fungus in slide culture closely resembles that of the *T. interdigitale* type. Dogs, cats, rabbits and guinea pigs may be infected; the granular types of the fungus are the more virulent.

Trichophyton rosaceum (*Trichophyton roseum, Ectotrichophyton megnini, Megatrichophyton megnini, Megatrichophyton roseum, Aleuriospora rosacea*): This fungus infects the epidermis, nails and hair follicles, usually without marked inflammation, and lesions are most common in the beard. It is common in the north of England and endemic in most of Europe; it has been found with some frequency in Philadelphia. The infection is spread through barber shops.

Infection of the hair is of the ectothrix type, the spores are arranged in chains and, in contrast to *T. mentagrophytes*, are large. The lesion on the skin is usually dry and scaly, and the fungus elements may be found in scrapings. The colony is velvety, pure white when young and gradually turning a pale rose with maturity while the under side becomes violet. Chlamydospores and conidia, *en grappe* and as thyrsi, are found in slide culture, but the morphology is not characteristic. Infection may be produced in the guinea pig.

Epidermophyton floccosum (*Epidermophyton inguinale, Epidermophyton cruris, Tri-*

chophyton inguinale, Trichophyton cruris, Closterosporia inguinalis, Fusoma cruris): This fungus is the cause of classic eczema marginatum (dhobie itch) and is ubiquitous in distribution though generally regarded as considerably more prevalent in the tropics. It may be disseminated in semi-epidemic form. It is also the cause of a minority of the intertriginous dermatophytoses of the feet. This fungus has never been observed to infect the hair follicles. The trichophytin reaction is practically always negative.

In scales taken from the advancing margin of the lesion hyphae and chains of spores may be found, in large amount when the scaling is profuse. Growth is relatively slow. The colony is characteristically an olive drab color, aerial mycelium is usually scant, and the surface is velvety and irregularly folded with radial furrows. Pleomorphic growth, manifested as the appearance of white tufts, appears relatively early. Blunt end fuseaux are present in groups in slide cultures of the fluffier strains and there are many chlamydospores, but structures other than raquet mycelium are seldom found. The fungus is only slightly pathogenic for the guinea pig.

Epidermophyton purpureum (*Trichophyton purpureum*): This fungus is closely related to *Epidermophyton* (*Trichophyton*) *rubrum* and is one of the common causes of intertriginous infection of the foot. It invades the skin to produce a typical psoriasis-like lesion, mildly inflammatory with the affected area dull red and scaly and the skin thickened. It is not infrequently found in onychomycosis, and a mild suppurative folliculitis may be produced in the beard. The fungus is found in the Far East and is common in the tropics and the southern part of this country. The trichophytin reaction is negative or faintly positive.

Fungus elements are rare in infected material. The colony is at first raised, fluffy and white. Radial grooves are formed and the typical rose-purple color at the back of the colony develops early and gradually diffuses into the colony to give a white center on a tinted background. Strain variation in the formation of aerial mycelium results in considerable variation in color as viewed from above. The morphology in slide culture is not characteristic, consisting largely of vegetative hyphae with occasional raquet mycelium, conidia, fuseaux and chlamydospores.

Endodermophyton concentricum (*Trichophyton concentricum*): This fungus is the commonest cause of *tinea imbricata* (*tokelau, Malabar itch*), a ringworm of the glabrous skin found in the South Pacific Islands, the Far East, and in India and Ceylon. Recently it has been reported from South America but apparently does not occur in this country. The closely related species, *Endodermophyton indicum* and *Endodermophyton tropicale*, are frequently found in Malaysia and Ceylon respectively. The name *E. tropicale* is used by many as a synonym of *E. concentricum.* The fungus grows between the superficial and deeper layers of the skin with little or no inflammation to produce a dry scaling lesion. The picture of the disease is highly characteristic; a circinate lesion is produced and as the margin advances, the process begins again in the center and similarly advances peripherally. With repetition concentric rings of white scales are formed. The lesions may be widespread over the body, those from different foci intermingling to produce a complex pattern. There is some disagreement as to whether the nails can be invaded.

Hyphae and spores may be found in the scales and the appearance is very similar to that of *Epidermophyton* scales. Primary cultures must be made in liquid media. Growth on maltose agar is scanty and the colony a light gray with a central knob. On glucose agar growth is profuse, the colony is wrinkled and grayish white, changing to amber or brown. The growth consists largely of sterile hyphae and is not characteristic.

Malassezia furfur (*Microsporon furfur, Monilia furfur*): This fungus is the causative agent of *pityriasis* (*tinea*) *versicolor*, a disease characterized by a benign macular eruption with scaling and a brownish discoloration of the affected skin. The inflammatory reaction is conspicuously mild. The fungus may be found in large numbers in the scaling epidermis as fragments of branched mycelium and clusters of spores. The latter are thought by some to be conidia arising from the mycelium. In spite of claims to the contrary, it is generally believed that the fungus has not been cultivated and it is, therefore. very poorly known. Both pityriasis versicolor and erythrasma are characterized by recurrences after they have been cleared up by treatment. The organisms seem to be ubiquitous, living on the skin surface of susceptible individuals.

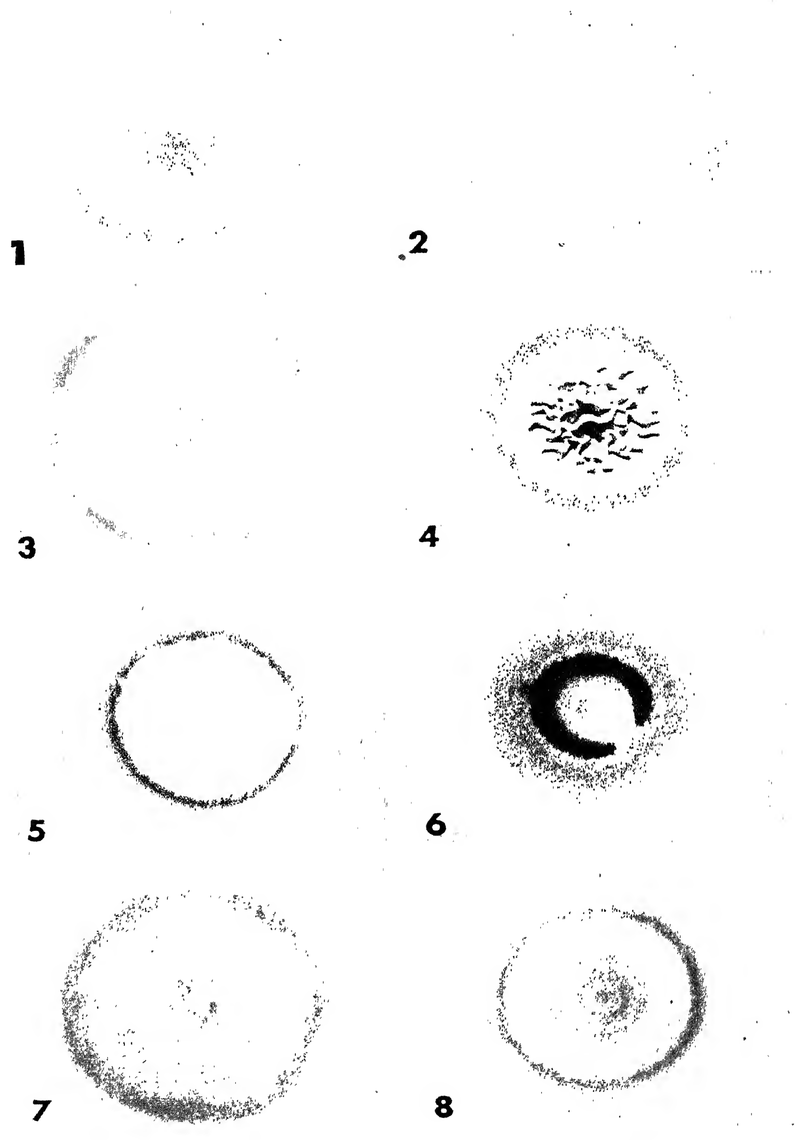


Fig. 167.—Colonies of some of the more common dermatophytes on Sabouraud's agar. *1*, *Microsporum audouini*. *2*, *Microsporum lanosum*. *3*, *Microsporum gypseum*. *4*, *Achorion schoenleini*. *5*, *Epidermophyton (Trichophyton) purpureum*. *6*, *Trichophyton violaceum*. *7*, *Trichophyton mentagrophytes* var. *granulosum*. *8*, *Epidermophyton floccosum*. Note the characteristic colors and gross morphology. × $\frac{3}{4}$.

Diagnosis.—As indicated earlier, classification of the dermatophytes is based largely on the clinical character of the infection and on colonial morphology on maltose-peptone agar. The differentiated structures developed in culture are, in some instances, of ancillary value. Fermentation reactions, emphasized by Castellani, are generally regarded as of little or no differential value. Animal inoculation is of minor importance unless the lesion is atypical and it is desirable to establish pathogenicity.

The probable cause of the disease can often be closely approximated on the basis of its clinical character; the usual associations are summarized in the accompanying table modified from Henrici.[1] Demonstration of the fungus by direct microscopic examination, however, is essential to establish the etiology and culture is often required for species identification.

The specimen material must be chosen with some care and taken in abundance for both microscopic examination and culture. In ringworm of

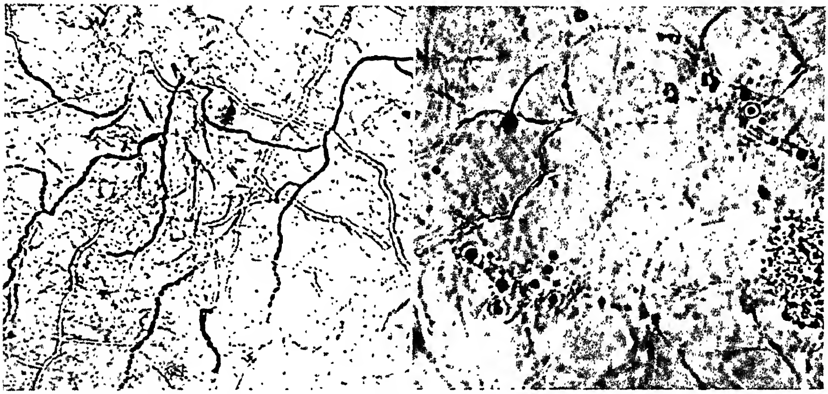


Fig. 168.—Skin scrapings from lesions of dermatophytosis. Left, hyphae and spores. Right, hyphae and epithelial scales. KOH preparation; × 450 (Rothman).

the scalp epilated stumps of hairs may be taken, and in addition scutula should be taken in favus and the contents of abscesses when the infection is suppurative. In infections of the smooth skin, scrapings from the scaly types should be taken from the margins, rolling toward the normal skin, and the tops of vesicles may be clipped with small scissors in the vesicular types. Macerated epithelium may be taken from intertriginous infections and nail scrapings and subungual hyperkeratotic masses from onychomycosis; the fungus elements are often more difficult to demonstrate microscopically in such specimens.

For direct microscopic examination the material is placed on a slide, a few drops of strong (20 to 40 per cent) sodium hydroxide solution added, covered with a cover glass, and the preparation heated slightly. Only mycelial elements and spores may be found in specimens. Permanent stained preparations may be made by mounting in Amann's medium, a lactic acid-glycerol-phenol solution containing cotton blue. As indicated

earlier, sections may be stained by Gram's method or with methyl green-pyronin.

Sabouraud's agar is the medium of choice for primary isolation as well as determination of colonial character. Inoculation should be relatively heavy and a number of plates prepared. Growth is relatively slow, usually ten days to three weeks are required, and the dermatophytes grow well at 30° C. Bacterial contamination may be a source of considerable difficulty, and it must be remembered that contamination with saprophytic fungi from air and dust readily occurs. The method of slide culture has been developed in large part by Henrici[1] and this and other methods of study of the fungi are described fully by him.

III. YEASTS AND YEAST-LIKE FUNGI

Yeasts are often defined as unicellular, nucleated organisms which reproduce by budding. Such a definition is, however, generally recognized

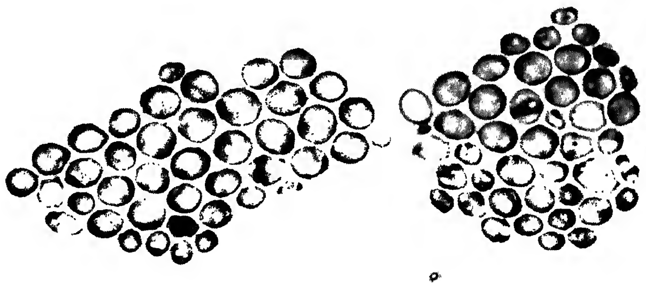


Fig. 169.—*Saccharomyces cerevisiae.* Smear from a pure culture. Methylene blue; × 1050.

as inadequate, in part because some yeasts produce by fission, in part because many, possibly all, produce mycelium under appropriate conditions, and in part because other fungi may exist in a unicellular, yeast-like form which reproduces by budding, *viz.*, the oidia described in the previous section. On the basis of sexual spore formation, some yeasts are ascomycetes, others are probably basidiomycetes, and still others have not been shown to have a sexual stage and are grouped with the Fungi Imperfecti. Clearly, then, the term "yeast" is of somewhat uncertain significance; as usually used it refers to those organisms which exist usually or predominantly in a yeast-like form.[84]

The yeasts are usually divided into three large groups, the basidiospore-forming yeasts or *Sporobolomycetaceae*, the ascospore-forming yeasts *Endo-*

[84] The genetics, cytology and classification of the yeasts have been reviewed by Henrici: Bact. Rev., 1941, 5:97.

mycetaceae, and the asporogenous yeasts *Torulopsidaceae*. The industrial yeasts are, perhaps, the most familiar of these. *Saccharomyces cerevisiae*, a member of the second group, is the common brewing yeast and occurs as two types, top yeasts which produce a vigorous evolution of carbon dioxide and are found in the froth on the surface of the fermenting mixture, and the bottom yeasts which sink to the bottom. Bread yeasts are usually top strains of *S. cerevisiae*. Another species of this genus, *Saccharomyces ellipsoideus*, is the common wine yeast, occurring naturally on grapes and in the soil of vineyards, and its varieties are named for the various types of wine which they produce. The mechanism of the alcoholic fermentation has been discussed elsewhere (p. 102). Still other yeasts are lactose-fermenters and are associated with the preparation of fermented milk beverages, such as kefir and koumiss, especially in southeastern Europe. Perhaps the commonest yeasts encountered as contamination in bacterial cultures and found growing on foods are the asporogenous

Fig. 170.—Giant colony of *Torula glutinis*, a saprophytic species, on Sabouraud's agar. × 2.

torulae; the pink or coral pigmented forms often observed are *Torula glutinis*.

In view of the ubiquitous distribution of yeasts, not only in air, dust and soil, but on the surface of the body and in the mouth, intestinal tract and vagina, it is not surprising that these forms have been found in a variety of pathologic processes. A great number of species have been described, most of them inadequately, in this connection. In many instances the yeast probably had no etiologic relation to the disease, and in others the same yeast has been repeatedly described as a new species to give rise to several synonymous names, and a very long list of "pathogenic" yeasts has accumulated.

Critical examination and consideration has now made it clear that only a very few species of yeasts are actually pathogenic for man and animals. These fall into two groups, the one containing the causative organism of

European blastomycosis or torula meningitis, commonly known as *Cryptococcus hominis* or *Torula histolytica*. As an example of the accumulation of "species" of pathogenic yeasts, Giordano[85] has listed sixty-eight synonyms for this organism. The second group is made up of the "medical Monilias," members of the subfamily *Mycotoruloideae* of the family *Torulopsidaceae*, which produce superficial infections of the skin and mucous membranes.

In addition to these a number of yeast-like fungi are considered in this section. These, together with the mold-like fungi discussed earlier, may be regarded as intermediate forms. The differentiation of these forms into "yeast-like molds" and "mold-like yeasts" implied by this separation is largely arbitrary.

PATHOGENIC YEASTS AND YEAST-LIKE FUNGI

Organism	Disease	Geographic Distribution
Debaryomyces neoformans	European blastomycosis, torula meningitis	Europe and U. S., probably ubiquitous
Blastomyces dermatitidis	American blastomycosis	U. S., possibly Europe and elsewhere
Candida albicans	Moniliasis	Ubiquitous
Coccidioides immitis	Coccidioidomycosis	Exclusively southwestern U. S.
Histoplasma capsulatum	Histoplasmosis	Probably ubiquitous, most cases reported from South America and U. S.

Debaryomyces Neoformans (Cryptococcus Hominis, Torula Histolytica)

In medical literature the term *blastomycosis* has been used very loosely to designate etiologically diverse infections in which budding cells are found in the tissues. The name itself is unfortunate in that yeasts are not "blastomycetes." Confusion arises largely between European blastomycosis, which will be considered here, and American blastomycosis, taken up in the following section.

Aside from moniliasis, two general types of yeast infection may be distinguished, the one the deep-seated cutaneous or subcutaneous infections which tend to become generalized, and the other infections of the central nervous system arising, as a rule, by metastasis from foci in the lungs. The first of these is known as European blastomycosis and the second as torula meningitis.

European Blastomycosis.—What was probably the first case of yeast infection of proven etiology was a fatal generalized infection observed by Busse and by Buschke[86] in 1893 which they called systemic blastomycosis.

[85] Giordano: Mycopathologia, 1938–9, *1*:274.

[86] *Cf.* Kolle, Kraus and Uhlenhuth: *Handbuch der pathogenen Mikroorganismen*. Gustav Fischer. Jena. 1928. Vol. 5, p. 321.

From primary ulcers on the face and neck the infection spread to the cervical lymph nodes, and the causative organism was isolated first from a secondary tibial abscess, then from the primary ulcers, and shortly before

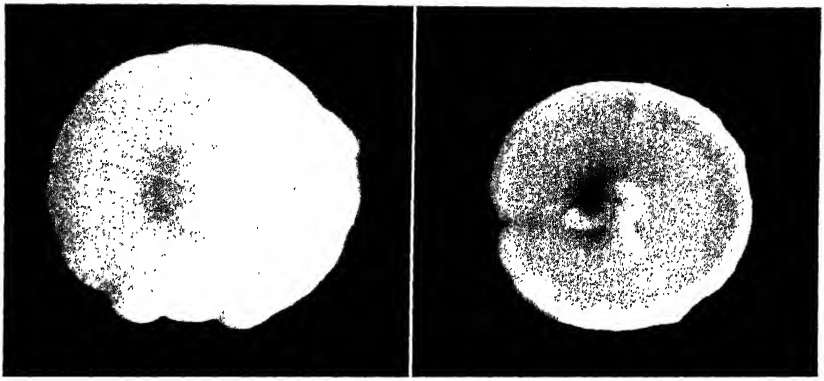


Fig. 171.—Giant colonies of torulae isolated from torula meningitis. Strain on left isolated by Shapiro and Neal, on right by Hirsch. Four weeks old, wort agar, natural size.

death from the blood stream. Other cases reported since are of this general type, characterized by deep-seated ulceration of the skin, sometimes granulomatous, and there may be infection of the viscera,[7] usually secondary.

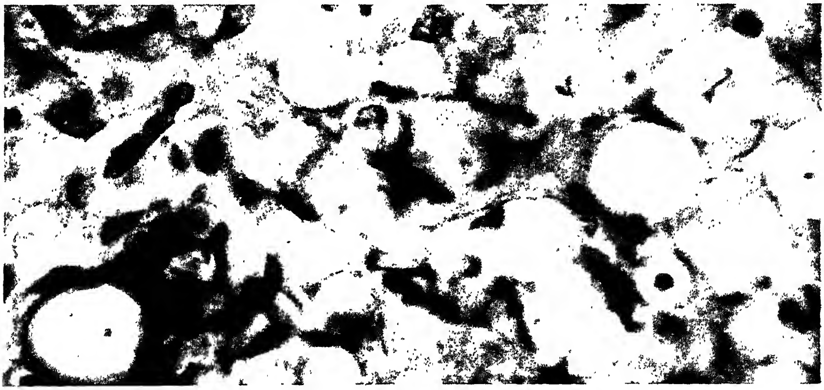


Fig. 172.—Torula meningitis in man. Section of the brain showing the growth of *Debaryomyces neoformans* in the tissues. Note the yeast-like cells embedded in masses of hyaline material which fill and distort the macrophages. Hematoxylin and eosin; × 950 (Humphreys).

involving the spleen, liver, kidneys and mesenteric lymphatics. Tumor-like masses are sometimes found and secondary infection of the lungs occurs with some frequency.

The microorganisms are found in exudates and in mucoid masses of gelatinous material as round to oval cells, 5 to 6 μ in diameter, surrounded by a mucilaginous sheath. The gelatinous material in which they may be embedded is evidently a product of the fungus. They are readily cultivated on most ordinary media as a smooth white or very light tan colony without distinguishing features.

Torula Meningitis.—A number of cases of meningitis of yeast etiology have been reported, the great majority from this country. The disease is the subject of a monograph by Stoddard and Cutler[87] who, in 1916, reported two cases and reviewed ten others. In recent years the disease seems to be becoming more common, or at least is reported more frequently.[88]

The clinical features of the disease set it off sharply from European blastomycosis for the infection is predominantly one of the central ner-

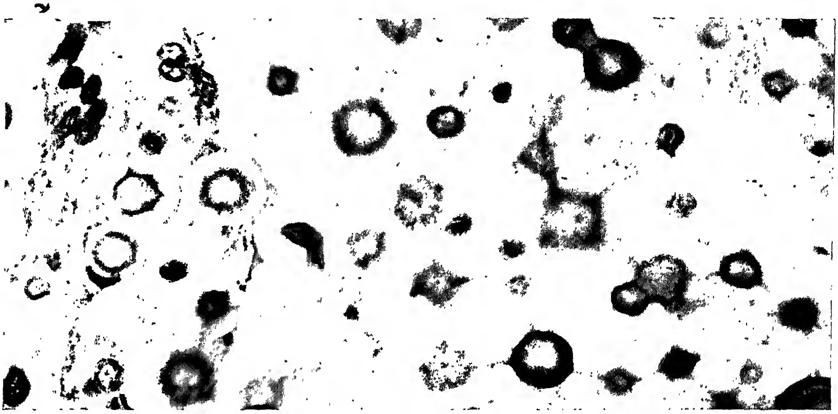


Fig. 173.—Torula meningitis in man. Growth of *Debaryomyces neoformans* in the cerebrospinal fluid. Note the budding cells, the stained capsules which give a double-contoured appearance, and the characteristic threads connecting the cells. Mucicarmine stain (staining the threads and capsules pink); × 950 (Humphreys).

vous system and the skin is seldom involved. The symptoms are those of brain involvement, especially intracranial pressure. Brain tumor may be closely simulated in some cases and the disease develops slowly, usually without febrile reaction or other signs of infection. The pathological picture is that of a chronic leptomeningitis with thickened meninges adherent to the cerebral cortex and showing diffuse or focal granulomatous lesions. The cerebral cortex is invaded in about half the cases; the lesions are sometimes granulomatous but more often cystic and there is little if any inflammatory reaction. The granulomatous lesions of both meninges and brain contain large accumulations of macrophages which phagocytose the

[87] Stoddard and Cutler: *Torula Infection in Man.* Monograph No. 6, Rockefeller Institute for Medical Research. 1916.

[88] *Cf.* Blair: Jour. Mental Sci., 1943, *89*:42.

fungus, while the cystic lesions consist of enormous numbers of yeast cells embedded in a gelatinous matrix.[89] The yeast is usually present in the spinal fluid in pure culture and may be observed in wet unstained preparations of the centifuged sediment.

The Causative Organism.—For some time the relationship of these clinically diverse infections was not realized, and the yeast of European blastomycosis was known as *Cryptococcus hominis* and that of torula meningitis as *Torula histolytica*. In 1934, however, Benham[90] showed that strains of the two yeasts were substantially identical, the difference in pathogenicity being one of degree only. Similar observations have been reported by others.[91] It seems established, therefore, that these diseases are caused by the same etiological agent and, furthermore, that only a single species of yeast is involved, observed strain differences having no more than variant status.

Until relatively recently this yeast was regarded as an asporogenous form properly grouped under the family *Torulopsidaceae*. Todd and Herrmann,[92] however, have described the formation of ascospores and their observations have been confirmed and extended to a considerable number of strains by others.[93] Spore formation has some unusual features which need not concern us here, but since single spores are formed by heterogamous conjugation, the organism is referred to the genus *Debaryomyces* of the family *Endomycetaceae*. Todd and Hermann[92] suggested the name *Debaryomyces hominis* but Henrici[84] and others have pointed out that the valid name is *Debaryomyces neoformans*.

Blastomyces Dermatitidis (American Blastomycosis)

The fungus disease known as American blastomycosis was first observed in Baltimore in 1894 by Gilchrist[94] and is sometimes called *Gilchrist's disease*. The great majority of cases have been found in this country, hence the name American blastomycosis, and the disease has been observed more often in the state of Illinois than in other states and is occasionally called *Chicago disease*. According to Martin and Smith[95] only two proved cases have originated outside the United States, one in Canada and one in England, though analysis of presumptive and inadequately described cases is difficult because of the usual failure to distinguish this disease from European blastomycosis.

The Causative Organism.—This fungus was isolated by Gilchrist and

[89] The clinical character of the disease and its pathology are discussed in some detail by Freeman: Jour. Psych. Neurol., 1931, *43:*236.

[90] Benham: Arch. Dermat. Syphilol., 1934, *30:*385; Mycologia, 1935, *27:*496; Jour. Inf. Dis., 1935, *57:*255.

[91] Lodder: Die Hefesammlung des "Centrallbureau voor Schimmelcultures" II Teil. Die Anaskosporogenen Hefen. Erster Hälfte. Amsterdam. 1934.

[92] Todd and Herrmann: Jour. Bact., 1936, *32:*89.

[93] Redaelli, Ciferri and Giordano: Boll. Sez. Ital., Soc. Internat. Microbiol., 1937, *I–II*, 1.

[94] *Cf.* Gilchrist: Johns Hopkins Hosp. Repts., 1896, *1:*269.

[95] Martin and Smith: Amer. Rev. Tuberc., 1939, *39:*275. This paper is a complete review of the literature to 1939 and a detailed analysis of 347 cases of the disease.

Stokes[96] from a second case of the disease and named *Blastomyces dermatitidis*. It is dimorphic, occurring only as a unicellular, budding, yeast-like form in the tissues, but as a mycelial form in culture. The unicellular form may be observed in sodium hydroxide preparations of pus or sputum. The cells are large, 8 to 10 μ in diameter, round or oval, and doubly contoured. The granular content of the cells distinguishes them from air bubbles or fat droplets and identification is practically certain if budding cells are found.

Colonial Morphology.—This unicellular morphology also occurs in cultures on blood agar, microscopic examination showing budding cells with a few rudimentary hyphae. The colony developing at 37° C. is wrinkled and waxy and somewhat similar to those of the tubercle bacillus in appearance. In culture on Sabouraud's agar the mycelial form appears. The colonial morphology is somewhat variable and Henrici[1] distinguishes three

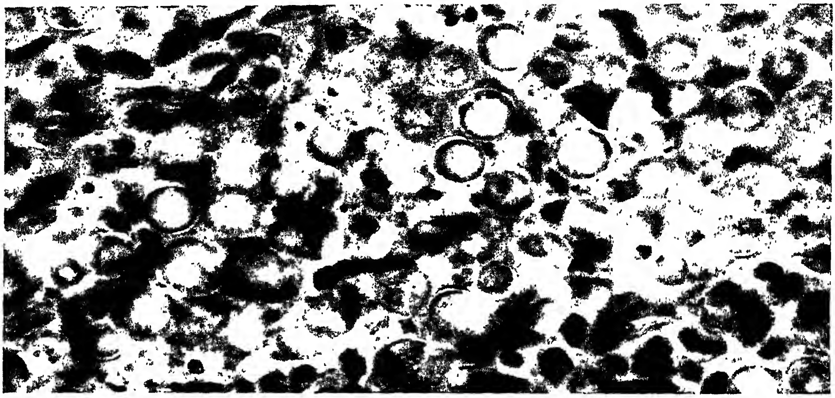


Fig. 174.—Blastomycotic pneumonia in man; infection with *Blastomyces dermatitidis*. Note the enormous numbers of yeast cells in the lung tissue and the variation in size and budding forms characteristic of active proliferation. Hematoxylin and eosin; × 1050 (Humphreys).

types. The "mealy" type is most often observed in primary isolation cultures and is similar to the growth on blood agar; microscopically the fungus is transitional between the unicellular and mycelial forms with many of the cells tending to form articulated chains. After one or two transfers the growth may assume a prickly surface, the prickles consisting of closely packed mycelial filaments which tend to become loose and cottony with continued incubation. The third type, sometimes observed on primary isolation and commonly found on laboratory cultures which are frequently transferred, is characterized by a white cottony growth with abundant aerial mycelium; in these colonies the unicellular form has completely disappeared and the growth consists of septate hyphae. Conidia are borne on lateral conidiophores but reports by some workers of the formation of asci have not been generally confirmed. The mycelial form reverts to

[96] Gilchrist and Stokes: Bull. Johns Hopkins Hosp., 1896, *7*:129.

the unicellular type in experimental infections or when cultivated on blood agar.

Classification.—The relationships of this fungus are somewhat uncertain though it is clearly not a "true yeast." Taxonomic considerations have led to the proposal of a number of different names; these include *Oidium dermatitidis*, *Gilchristia dermatitidis*, *Cryptococcus gilchristi*, *Mycoderma dermatitidis*, *Zymonema dermatitidis* and others. Systematists object to all these for one reason or another and none has been generally accepted. In spite of the fact that, strictly speaking, the generic name *Blastomyces* is improper from the taxonomic point of view, it continues to be generally used and the fungus is known as *Blastomyces dermatitidis*.

Pathogenicity.—Primary infection occurs most commonly in the skin and the lesions begin as a small firm papule about which secondary nodules develop which enlarge with coalescence. The process breaks down in the center and becomes suppurative with discharge of pus through small fistulae. The inflammatory reaction is granulomatous with connective tissue formation, proliferation of the epithelium, mononuclear infiltration and sometimes giant cell formation. The fungus is found in the pus of minute miliary abscesses which are a characteristic feature of the pathology. As the disease progresses, therefore, a large elevated mass of tissue, with an irregular ulcerated surface, is formed and oozes pus from multiple small openings upon pressure. The resemblance to epithelioma or tuberculous ulcer is sometimes striking. Lesions with pronounced epithelial proliferation simulate tuberculosis verrucosa. The process spreads slowly through the subcutaneous tissues and often becomes generalized by way of the bloodstream.

Primary infection of the lungs is not uncommon and often closely resembles tuberculosis clinically with cough, pain in the chest, weakness, sometimes hemoptysis, and productive sputum late in the disease. The pathologic picture is variable in that there may be focal or diffuse consolidation, and the abscesses may be miliary or there may be larger nodules. Cavitation occurs but is limited to small areas. The microscopic picture also resembles tuberculosis and sometimes it is difficult to differentiate unless the budding cells are found.

With generalization from primary foci of infection, multiple small abscesses of hematogenous origin occur throughout the body. They are most common in the subcutaneous tissues and differ from the primary skin lesions in that they develop without pain or marked erythema, are soft and evacuate considerable quantities of pus when opened. In contrast to the primary skin lesions, which most commonly occur on exposed parts of the body, the secondary subcutaneous abscesses are usually found in covered areas. Secondary abscesses also commonly develop in the viscera and muscles and under the periosteum. There is a septic febrile reaction and the generalized disease is probably almost always fatal.[97]

The source of infection is not clear. Very often the primary skin lesion develops from a wound infection and a large proportion of the cases have

[97] The histopathology of blastomycosis is discussed by Baker: Amer. Jour. Path., 1942, *18*:479.

been in farmers. Males are much more frequently attacked and the incidence is higher in the twenty to fifty age group. It is possible that the fungus is a free-living form which, like *Sporotrichum*, occasionally infects man. One case of direct transmission, by autopsy infection, is known.

Pathogenicity for Animals.—The inoculation of experimental animals is not uniformly successful and has no diagnostic utility. According to Spring[98] mice are more susceptible than guinea pigs, and rabbits are almost completely resistant. Small caseous nodules develop on the peritoneal surfaces of intraperitoneally inoculated animals, and the type of tissue reaction varies with the resistance of the animal and the virulence of the strain of fungus from frank abscess formation to tubercle-like lesions. Generalized blastomycosis has been demonstrated in a naturally infected dog and the fungus found to be identical with typical strains of human origin.[99]

Immunity.—There appears to be little or no effective immunity in man to spread of the infection since generalization is common. Complement-fixing antibodies are produced, however, and the titer is related to the severity of the infection. A hypersensitivity to the cell substance of the fungus occurs also and is manifested as a delayed tuberculin-like response to intradermal inoculation of suspensions of killed cells. The hypersensitivity is of practical importance in that it contraindicates the therapeutic use of iodides; desensitization may be accomplished with vaccine. It has not as yet been possible to demonstrate an effective immunity to the experimental infection in laboratory animals.

Diagnosis.[100]—As indicated above, the yeast-like unicellular form of the fungus can be demonstrated by direct microscopic examination of pus or sputum mounted in sodium hydroxide. Positive complement-fixation and skin tests are of value in pulmonary and systemic infections. An unequivocal diagnosis can be established, however, only by isolation and identification of *Blastomyces dermatitidis*. This is usually not difficult if the specimen is not too heavily contaminated with bacteria; the fungus grows readily on Sabouraud's agar, the colonial morphology varying as indicated earlier.

Candida Albicans (Monilia Albicans)

The fungi which make up the group of "medical monilias" are yeast-like organisms which are usually found in the budding unicellular stage. Mycelium is formed only under semi-anaerobic conditions or in submerged cultures, and may be found in the tissues. When cultured on agar in the usual way, the colonies are smooth and waxy and on microscopic examination are found to consist entirely of budding, yeast-like cells. Gelatin stab culture is a convenient method of differentiating these forms from the yeasts for, while the surface growth is yeast-like, growth along the line of inoculation includes the extension of radiating filaments into the medium; these consist of mycelium from which budding cells arise. The same type

[98] Spring: Jour. Inf. Dis., 1929, *44*:169.

[99] Foshay and Madden: Amer. Jour. Trop. Med., 1942, *22*:565.

[100] For a detailed discussion of the usual methods of laboratory diagnosis see Martin: Jour. Inf. Dis., 1935, *57*:291; Martin and Smith: Jour. Lab. Clin. Med., 1936, *21*:1289.

of growth may be observed on agar plates by cutting deeply into the medium with the inoculating needle and examining the growth through the bottom of the plate with the low power. In general, under conditions favorable for growth and rapid multiplication, the yeast-like form predominates, while mycelium appears under less favorable conditions.

The first description of a fungus of this type occurring in disease was that of Langenbeck in 1839 who found it in patches on the mucous membranes of the mouth and elsewhere at autopsy. The organism was named *Oidium albicans* by Robin four years later. The generic name *Monilia* has come to be commonly used and the disease is usually termed *moniliasis*. The moniliases are usually superficial infections of the mucous membranes, the intertriginous areas and the nails, but pulmonary moniliasis is known and localized infections sometimes result in a generalized infection by hematogenous spread, especially in hosts debilitated by some other disease.

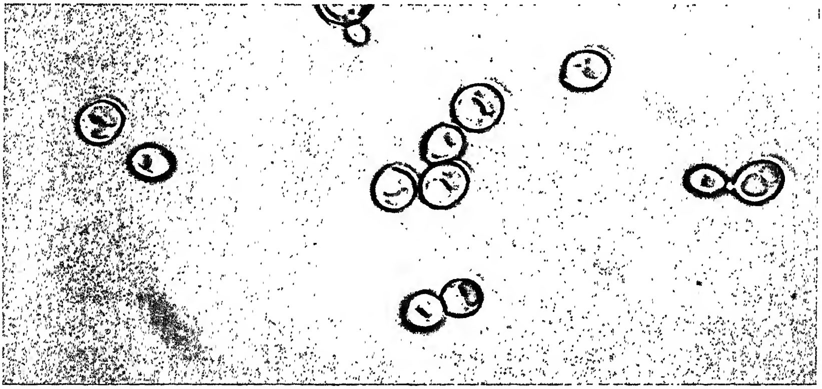


Fig. 175.—Unstained preparation of the yeast-like form of *Candida albicans*. Oblique lighting. Note the budding cell and close resemblance to the true yeasts. × 1050 (Rothman).

The Causative Organism.—In addition to the original *Monilia albicans*, a considerable number of species of *Monilia* have been described including *Monilia krusei*, *Monilia parakrusei*, *Monilia tropicalis*, *Monilia stellatoides* and others. In fact, a large proportion of current literature on these organisms is devoted to their differentiation, identification and classification. Differentiation is made on the basis of various cultural characters such as pellicle formation in liquid media, gelatin liquefaction and the like, and Castellani[101] has especially emphasized differential fermentations. The last is the most practical laboratory method but there is some disagreement as to the constancy of these characteristics. The various strains do not make up an immunologically homogeneous group but are closely related and show pronounced cross reactions.[102] It is generally

[101] *Cf.* Castellani: Jour. Trop. Med. Hyg., 1937, *40*:293.
[102] Martin: Amer. Jour. Trop. Med., 1942, *22*:295.

admitted, however, that there is no marked correlation of the various differential characters, and that species so differentiated are not associated with any particular type of infection, *i.e.*, the same species may cause a variety of disease processes and different species are found in the same sort of lesion. A further complicating factor is the inherent variability of these fungi; smooth, rough and transitional forms are observed, sometimes occurring simultaneously in the same patient. While the question of the plurality of species is by no means closed, the differentiation of a considerable number of species appears to be of limited value and justified only in part.[103] For the present, therefore, we shall assume that all the pathogenic monilias constitute a group of varieties of the single species, *Monilia albicans*.

Considerable interest has attached also to the proper generic name for these fungi. As indicated above, *Oidium* was used very early. A number

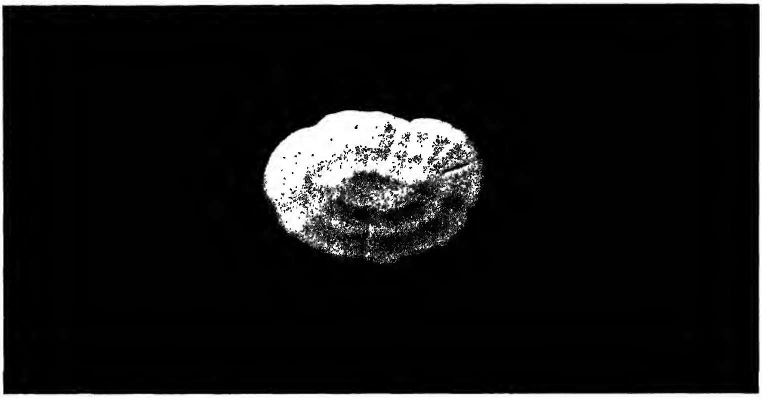


Fig. 176.—Giant colony of *Candida albicans* on Sabouraud's agar. Note the smooth creamy growth characteristic of yeasts. × 3.

of old generic names have never been widely used. It is generally recognized that *Monilia*, commonly used in medical literature, is a misnomer, for these yeast-like fungi are quite different from those included by the mycologist in the genus *Monilia* Persoon 1797. The name *Mycotorula* has been suggested in recent years, but the name *Candida* is probably preferable and in any case is coming into common use. The organism is, therefore, *Candida albicans* and, if other species are differentiated, they are properly species of *Candida*.

Pathogenicity.—These fungi are commonly present in the mouth, vagina and intestinal tract in normal persons and are probably not highly virulent with respect to initiation of the infectious process. In fact, as in many of the mycoses, there is frequently a history of a debilitated con-

[103] See Langeron and Guerra: Ann. Parasitol., 1938, *16*:36, 162, 429, 481. These authors review the literature critically, give a detailed discussion of the methods of differentiation, and recognize seven species of the genus.

dition or other predisposing factors. While in some instances in which they are associated with the development of a pathologic process there is some doubt as to their etiologic rôle, in many others it is clearly evident that they are primary invaders and responsible etiologic agents.

Infections of the Mucous Membranes.—Moniliasis of the mucous membranes is known as *thrush* and is one of the common mycoses. Intra-oral thrush, a superficial infection of the mucous membranes of the mouth, is the type most frequently observed and is considerably more common in nursing infants and children than in adults. In the past it has occurred in severe epidemic form in institutions such as foundling asylums but such epidemics are observed less often now. Henderson[104] has reported an incidence of 6.3 per cent in infants in the Simpson Maternity Hospital, Edinburgh, in 1940; strict isolation procedures reduced this to 3.7 per cent in 1942. Thrush occurs more frequently in bottle-fed than in breast-fed

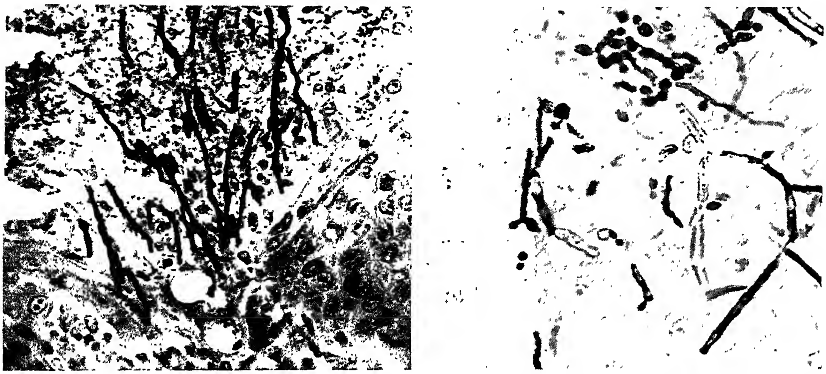


Fig. 177—Moniliasis: invasion of the epithelial layer of the esophagus by *Candida albicans*. Left, beginning invasion of the superficial layer showing mycelium and yeast-like cells; note the gram-positive micrococci accompanying the fungus; × 600. Right, invasion of the deeper tissue showing both mycelium and yeast-like cells penetrating the epithelium; × 950. Gram stains (Humphreys).

babies, and the lesions not infrequently spread to the pharynx and even the esophagus. In adults thrush generally occurs terminal to wasting diseases such as tuberculosis and cancer; dryness of the mouth associated with prolonged coma or near coma appears to favor infection. It also occurs with some frequency as a mild vaginal infection in pregnant women which may or may not be associated with infection of the anus.

In the majority of cases the infection is mild and remains localized. Occasionally it may spread to other mucous membranes and to the skin with the development of a generalized cutaneous eruption and intertriginous lesions, and such cases are sometimes fatal. The most common predisposing malady for cutaneous moniliasis is diabetes. Hematogenous spread with metastasis and abscess formation in the viscera has been observed.

[104] Henderson: Edinburgh Med. Jour., 1943. *50*:535.

The lesions appear as soft, whitish patches, composed largely of fungus growth. They are readily removed to leave an eroded surface. There is some resemblance to a diphtheritic membrane and lesions in the throat and on the tonsils have no doubt been mistaken for diphtheria. However, it is much easier to separate the membrane in moniliasis. On microscopic examination of the membranous material in wet preparation, the observation of a tangled mass of segmented mycelium admixed with budding yeast-like cells, desquamated epithelium and leucocytes establishes the diagnosis. The fungus may be cultivated on Sabouraud's agar but isolation in pure culture is more readily accomplished with dextrose tartaric acid agar. The isolated fungus must be differentiated from yeasts.

Dermatomoniliasis.—*C. albicans* is also causally associated with eczema-like lesions of the moist skin similar to those produced by *Epidermophyton floccosum.* Infection of the folds between the fingers, *erosio interdigitalis,* is more common than dermatophytosis of this region, and is found most often in those whose hands are frequently wet. *Perleche*, an infection of the angles of the mouth, is another form of intertrigo produced by these fungi. *Moniliids*, sterile vesicular or exudative lesions which appear on the hands secondary to a focus of infection elsewhere, are analogous to trichophytids and a result of hypersensitivity.

The nails, chiefly of the fingers, are also attacked by *C. albicans* with the production of chronic paronychia. The condition is differentiated clinically from onychomycosis by retention of the luster of the infected nail and the absence of yellowish discoloration, crumbling and thickening of the soft tissues. The nail shows transverse ridges and eventually becomes thickened, distorted and brownish in color. Like interdigital infections, the infection is found most often in those whose hands are often wet.

On microscopic examination of nail and skin scrapings cleared in 10 per cent sodium hydroxide, hyphae may be present together with the budding yeast-like cells.

Bronchomoniliasis.—There appears to be a distinct type of moniliasis of the respiratory tract which was first reported by Castellani[105] from Ceylon. It has been observed in Europe and this country but is apparently more common in the tropics. A number of moniliae have been isolated, all closely related, but that variety known as *Candida tropicalis* has been most often found. According to Castellani, two types of the disease occur; the mild form, a chronic bronchitis characterized by dyspnea and cough, is afebrile, while the severe form is similar to tuberculosis and usually fatal.

The fungi may be demonstrated by direct microscopic examination and by culture but such findings must be interpreted with caution, for the organism is frequently found in the sputum in other diseases, especially tuberculosis. It is recommended that the throat be cleansed by gargling before the sputum is collected and other contamination avoided. Strains isolated from cases of bronchomoniliasis are pathogenic for rabbits, producing tubercle-like nodules within two or three weeks after inoculation into the lungs.

[105] Castellani: Lancet, 1922, *i*:13; Military Surgeon, 1925, *57*:113.

Coccidioides Immitis

Coccidiomycosis, coccidioidomycosis or coccidioidal granuloma was first observed in Argentina in 1892 by Posados[106] and by Wernicke;[107] two years later it was described independently in California by Rixford.[108] The causative organism was thought to be a protozoan and named *Coccidioides immitis.* Ophuls and Moffitt[109] showed, by culture, that it is a fungus but this does not invalidate the name. Though originally reported from South America, the disease appears to be rare there and is most prevalent in the San Joaquin Valley in California and in the dry regions of the southwestern United States; it seems to be unknown elsewhere.

The Causative Organism.—Like a number of the other pathogenic fungi, *C. immitis* is dimorphic, and the parasitic stage within the tissues is morphologically different from that assumed in culture.

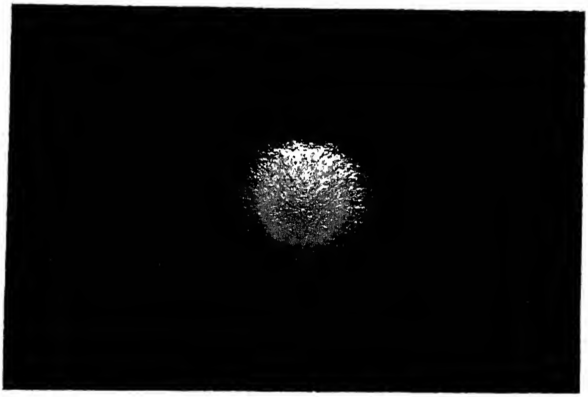


Fig. 178.—Colony of *Coccidioides immitis* on blood agar. Isolated from a fatal case of coccidioidomycosis. Natural size.

The Parasitic Stage.—This fungus differs markedly from the other yeast-like fungi in that it never reproduces by budding, and it differs from all the other pathogenic fungi in that it reproduces within the tissues exclusively by a process of endogenous spore formation. The newly liberated spores are small mononucleate spheres 1 to 3 μ in diameter and appear as a central, deeply stained mass of protoplasm surrounded by a double-contoured capsule. The cell enlarges, soon becoming multinucleate, and eventually reaches a diameter of 50 to 60 μ. A central vacuole appears early and in later stages occupies a large part of the cell, the protoplasm appearing as a thin peripheral layer. The peripheral protoplasm becomes vacuolated and an indefinite number of multinucleate protospores become delimited by the formation of cell walls; the protospores in turn subdivide to form

[106] Posados: Ann. Circ. Med. Argentina, 1892, *15:*585.
[107] Wernicke: Centralbl. f. Bakt., 1892, *12:*859.
[108] *Cf.* Rixford and Gilchrist: Johns Hopkins Hosp. Repts., 1896, *1:*209, 253.
[109] Ophuls and Moffitt: Philadelphia Med. Jour., 1900, *5:*1471.

spores and the entire cell assumes the functions of a sporangium. With rupture of the cell wall the mature spores are liberated and the developmental cycle begins again.[110] All of these forms may be observed in the tissues and in pus, though recently disseminated spores are difficult to demonstrate.

The Saprophytic Stage.—The fungus will grow on a variety of sugar-containing media but Sabouraud's agar is the medium of choice. It grows best at 37° C. under aerobic conditions. On artificial culture media the growth is that of a mold. The colony may be smooth and waxy when young but aerial mycelium is soon formed and it becomes gray or brownish in color. Emmons[110] has pointed out that, though it is often stated that the aerial hyphae break up into chlamydospores, the spores are borne on differentiated conidiophores and should be termed conidia or oidia. Spirals are frequently observed.

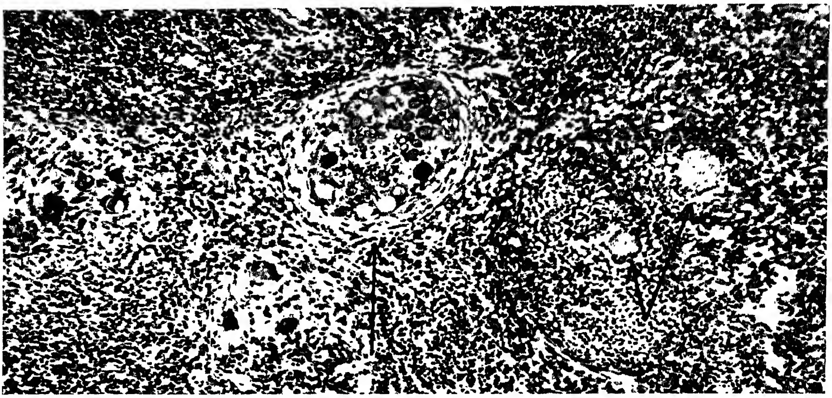


Fig. 179.—Coccidioidomycosis in man. Section of spleen showing a large lesion of the chronic type and two smaller lesions characteristic of the acute type of infection. In the latter sporulation is complete and the spores are beginning to spread into the surrounding tissue. Hematoxylin and eosin; ×125 (Humphreys).

Pathogenicity.—For many years infection with *C. immitis* was known only as coccidioidal granuloma, a severe disease in which the case fatality rate was thought to be 90 per cent or more. Infection with this fungus is, however, much more prevalent and less severe than had been supposed. Dickson[111] has observed healed lesions of coccidioidal granuloma at autopsy of persons dying of other causes, and Cox and Smith[112] have reported similar findings and produced arrested lesions in white rats. Furthermore, it was shown by Dickson[113] in 1937 that the disease may take a

[110] Nuclei were first described by Emmons in a recent review which includes complete descriptions of both parasitic and saprophytic stages of the organism as well as a discussion of the disease. *Cf.* Emmons: Mycologia, 1942, *34:*452.

[111] Dickson: Arch. Int. Med., 1915, *16:*1028.

[112] Cox and Smith: Arch. Path., 1939, *27:*717.

[113] Dickson: Calif. & Western Med., 1937, *47:*151. See also Arch. Int. Med., 1937, *59:*1029; Amer. Rev. Tuberc., 1938, *38:*722; Jour. Amer. Med. Assn., 1938, *111:*1362.

second form, a benign, acute, self-limited respiratory infection, in addition to the chronic, progressive granulomatous disease. He suggested that these be designated primary and secondary or progressive coccidioidomycosis respectively and these terms are now coming into general use.

Primary Coccidioidomycosis.—The infection is acquired by inhalation of the spores, and the disease varies in severity in recognized cases from that of a common cold to cases resembling influenza with pneumonia, cavitation and high fever. In a small proportion of the cases, possibly 5 per cent, an exanthem like erythema nodosum occurs and the disease is recognized as "the bumps," desert rheumatism, Valley fever, San Joaquin fever, etc. Minor "epidemics" may occur such as that described by Davis, Smith and Smith[114] in which seven of fourteen persons who spent two days on a desert field trip near the San Joaquin Valley became infected. The largest single outbreak yet recorded was reported by Goldstein and Louie.[115] Several

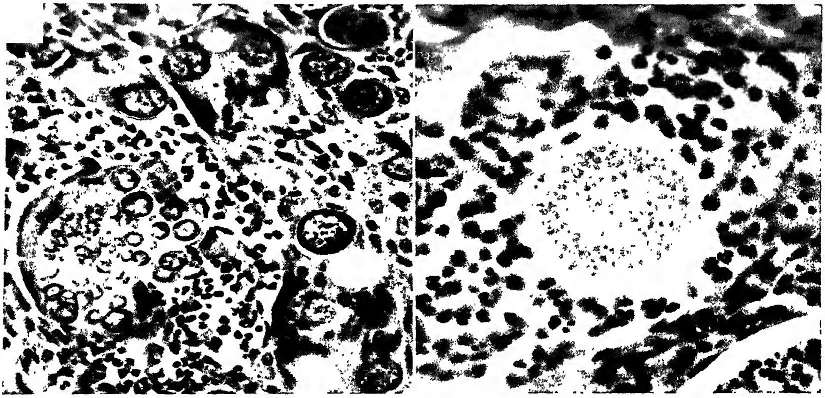


Fig. 180.—The chronic and acute types of lesion in coccidioidomycosis in the same section shown in Fig. 179, but under higher magnification. Left, the chronic type of lesion showing many mature cells of *Coccidioides immitis:* × 475. Right, the active type of lesion showing a mass of spores beginning to spread in the tissue. Hematoxylin and eosin; × 710 (Humphreys).

thousand soldiers were exposed during military training in a desert endemic area, and of these seventy-five contracted the disease, All but one made rapid progress to complete recovery, but the remaining individual developed a generalized infection and coccidioidal granuloma. Spontaneous recovery is the rule and only rarely does the disease progress to the secondary type.

Secondary or Progressive Coccidioidomycosis.—The progressive type of infection results in cutaneous, subcutaneous, visceral and osseous lesions. The cutaneous type closely resembles blastomycosis but is a much more severe disease with fever and a greater tendency to hematogenous spread. Elsewhere the lesions closely resemble those of tuberculosis and, in fact,

[114] Davis, Smith and Smith: Jour. Amer. Med. Assn., 1942, *118:*1182.
[115] Goldstein and Louie: War Med., 1943, *4:*299.

differentiation from that disease may often be possible only by demonstration of the fungus. It is not known whether the progressive type of disease results from a new infection or reactivation of arrested primary lesions.

Pathogenicity for Animals.—The disease occurs naturally in domestic animals including cattle, sheep and dogs[116] and also in certain wild rodents[117] in endemic areas. The latter include three species of pocket mice, *Perognathus baileyi*, *P. penicillatus* and *P. intermedius*, the kangaroo rat, *Dipodomys merriami*, and the grasshopper mouse, *Onychomys torridus*. Experimental animals are readily infected; both rabbits and guinea pigs are susceptible to intraperitoneal inoculation.

Immunity.—Since it is now apparent that infection with *C. immitis* can take a mild form from which spontaneous recovery is the rule, that arrested lesions occur with some frequency, and that the case fatality rates in the severe progressive type of disease are not as high as once thought, it is indicated that there is, on the one hand, an appreciable natural resistance to infection, and on the other, an effective immune response.

The immune response is manifested in part as the development of a hypersensitivity to the parasite. *Coccidioidin* is, like tuberculin (OT), prepared from liquid cultures of the parasite, but there is some difficulty in reproducing potencies. Hassid, Baker and McCready[118] have described an immunologically active polysaccharide which appears to make up at least a part of the active principle, producing both skin reactions and precipitin tests. Coccidioidin is inoculated intradermally, a positive reaction appearing in twenty-four to forty-eight hours. The hypersensitivity appears in a few days to a few weeks after infection, recent infections and severe infections resulting in stronger reactions than old infections and mild infections respectively. The test appears to be highly specific though there is as yet some disagreement as to its interpretation.[119]

Diagnosis.—The laboratory diagnosis of coccidioidomycosis is dependent upon the demonstration of the parasite, the diagnostic value of a positive coccidioidin reaction being somewhat uncertain. As indicated earlier, it may be found on direct microscopic examination of pus, spinal fluid and tissues, but may be difficult to demonstrate in the sputum. Lactophenol-cotton blue or Mallory's eosin and methylene blue may be used for staining. Though *C. immitis* grows without difficulty on sugar-containing media, it has been cultured in a surprisingly small proportion of cases; in 432 cases studied by Smith,[120] the fungus was recovered from only 22 per cent of the patients.

Epidemiology.—In the endemic areas of the southwestern United States the proportion of reactors to the coccidioidin test is high, ranging from 16 to 90 per cent.[121] The prevalence of infection in these regions ap-

[116] Beck, Traum and Harrington: Jour. Amer. Vet. Med. Assn., 1931, *78*:490; Davis, Stiles and McGregor: *ibid.*, 1937, *91*:209; *ibid.*, 1938, *92*:562; Farness: *ibid.*, 1940, *97*:263.

[117] *Cf.* Emmons and Ashburn: Pub. Health Repts., 1942, *57*:1715.

[118] Hassid, Baker and McCready: Jour. Biol. Chem., 1943, *149*:303.

[119] *Cf.* Stewart and Kimura: Jour. Inf. Dis., 1940, *66*:212.

[120] Smith: Amer. Jour. Pub. Health., 1940, *30*:600.

[121] Farness: Jour. Amer. Med. Assn., 1941, *116*:1749.

pears, therefore, to be much higher than suspected earlier. A very large proportion of these reactors have no history of coccidioidomycosis—Smith[120] estimates that only 5 per cent of infected persons show sufficiently pronounced clinical symptoms to be detected and diagnosed.

Though, as indicated earlier, the disease occurs in domestic animals and certain wild rodents, there is no evidence of direct transmission of the infection from animals to man. It seems likely both man and animals are infected by the inhalation of spores contained in dust; there is frequently a history of exposure to dust storms in human cases and the fungus has been isolated directly from dust.[122] *C. immitis* is probably not, however, a saprophyte which infects animals and man by chance, but rather a parasite, the reservoir of infection of which appears to be wild rodents that discharge spores in the feces and thus contaminate the soil resulting in transmission of the fungus to both other wild rodents and man.[123]

Paracoccidioidomycosis.—The disease known as paracoccidioidal granuloma or South American blastomycosis occurs in Brazil but has not been observed in this country. The causative organism is *Paracoccidioides brasiliensis* which, despite its name, is not closely related to *C. immitis*. It is a yeast-like fungus, proliferating by budding in the tissues, and grows in a compact cerebriform colony on Sabouraud's agar, eventually producing a white aerial mycelium.

The disease is clinically similar to coccidioidomycosis and blastomycosis. The fungus enters the body via the mouth to produce ulcerative granulomatous lesions which spread to the tongue, lips and nose. The infection spreads via the lymphatics to produce lesions of the viscera and often pulmonary involvement. It is usually fatal.

Diagnosis is established by microscopic demonstration of the multiple budding cells (in contrast to the singly budding cells of *Blastomyces dermatitidis*) in material from the lesions, or by culture.

Histoplasma Capsulatum

This organism was discovered in 1906 by Darling,[124] who observed it in sections of tissues taken post mortem from three cases of what appeared to be visceral leishmaniasis and named it *Histoplasma capsulatum*. The first case in the United States was reported in 1926. The disease is reviewed by Meleney[125] who summarized 32 cases known to 1940, all of which were American and 24 of which were found in the United States. Since then other cases have been reported, including one from England by Derry, Card and Wilson[126] and one from Africa by Simpson and Barnetson.[127] The increasing number of cases reported in recent years is no doubt a consequence of more general interest in the disease and suggests that it is more common than has been thought.

122 *Cf.* Emmons: Pub. Health Repts., 1942, *57*:109.
123 *Ibid.*,1943, *58*:1.
124 Darling: Jour. Amer. Med. Assn., 1906, *46*:1283.
125 Meleney: Amer. Jour. Trop. Med., 1940, *20*:603.
126 Derry, Card and Wilson: Lancet, 1942, *ii*:224.
127 Simpson and Barnetson: Jour. Path. Bact., 1942, *54*:299.

The Causative Organism.—The organism appears in the tissues as a small, oval, encapsulated yeast-like cell 3 to 5 μ in diameter. Larger strains, 10 μ in diameter, have been found in two cases and described as another species, *Histoplasma pyriforme*, but the implied difference or relationship is highly uncertain. In stained preparations the central stained mass is surrounded by a clear zone and the refractile capsule. The organism is usually intracellular, in the mononuclears in the peripheral blood and in the macrophages elsewhere, especially those of the bone marrow and spleen. Because of this characteristic intracellular position of the parasite, the disease is sometimes called *reticulo-endothelial cytomycosis* or simply *cytomycosis*.

The organism was cultivated in 1934 by DeMonbreun[128] from the blood during life and the spleen at autopsy of one case, and by Hansmann and Schenken[129] from biopsy material from another. It grows in the yeast-like

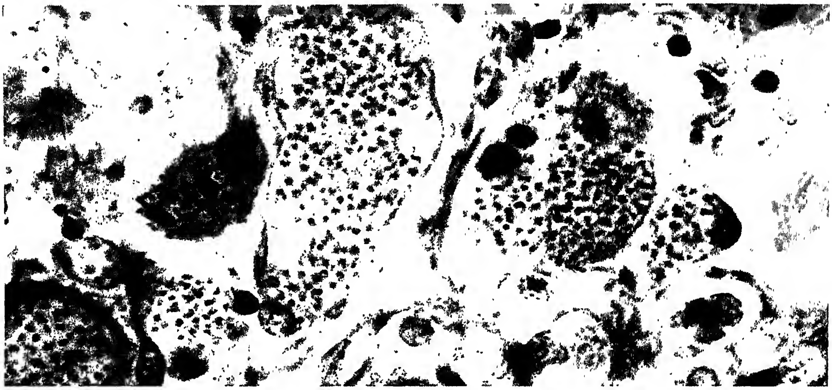


Fig. 181.—*Histoplasma capsulatum.* Yeast-like cells in the macrophages of the bone marrow in systemic histoplasmosis. Hematoxylin and eosin; × 1000 (Humphreys).

form seen in the tissues in sealed blood agar cultures, but on Sabouraud's agar a mycelial form is assumed and the colonies are mold-like, white and cottony with aerial mycelium. Chlamydospores are formed in abundance, first smooth and pyriform, but as they mature they become larger, 7 to 15 μ in length, thick-walled and tuberculate with finger-like protuberances sometimes as much as 6 μ long. Conant[130] has studied these developmental stages in detail and has been able to convert the mycelial stage to the yeast-like form by cultural methods. The fungus has been regarded by some as closely related to *Paracoccidioides brasiliensis*.

Pathogenicity.—Meleney[125] has differentiated four general types of disease produced by *H. capsulatum*. The clinical picture is sometimes one of a systemic febrile disease with splenomegaly, septic temperature curve, anemia and leucopenia, simulating kala-azar. A second type is distin-

[128] DeMonbreun: Amer. Jour. Trop. Med., 1934, *14*:93.
[129] Hansmann and Schenken: Amer. Jour. Path., 1934, *10*:731.
[130] Conant: Jour. Bact., 1941, *41*:563.

guished by the predominance of lymphatic involvement and resemble Hodgkin's disease, lymphosarcoma, leukemia or aplastic anemia. Or pul monary symptoms may predominate with cavitation and fibrous adhesions of the pleurae, the infection sometimes complicating tuberculosis. A fourth type is characterized by ulcerative skin lesions; ulceration also occurs in the intestine.

Though it is sometimes implied that histoplasmosis is always a generalized infection of the macrophage system, the parasites being especially numerous in tissues rich in these cells such as the spleen and bone marrow, this is by no means generally true. In most cases there are nodules or extensive areas of necrosis in one or more organs and in some instances only a single organ, such as the adrenals, has been infected. These necrotic lesions usually consist of a central area of necrosis surrounded by granulation tissue containing large numbers of macrophages and ingested para-

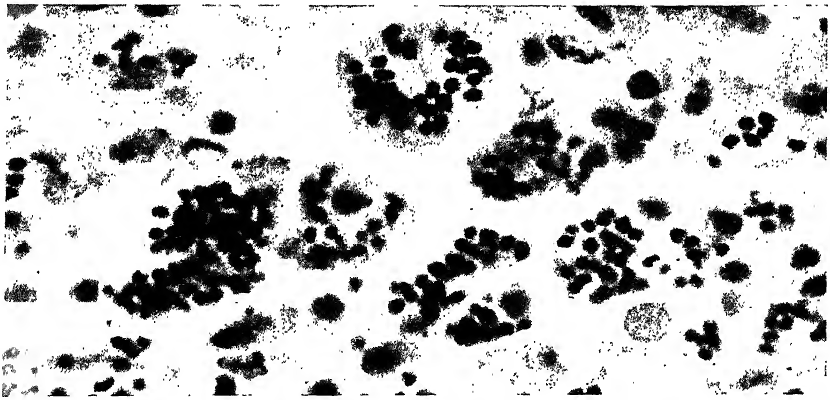


Fig. 182.—*Histoplasma capsulatum*. Yeast-like cells in the suprarenal gland in systemic histoplasmosis. Gram stain; × 1350 (Humphreys).

sites. In some instances the parasite has been found to be limited to such areas, while in others it is widely distributed in the macrophage system as well.

Pathogenicity for Animals.—A naturally occurring infection in a dog has been described by DeMonbreun[131] and was experimentally transmitted to puppies by oral and parenteral inoculation. Similar infections of ferrets and mice have been reported though not proved to be histoplasmosis. Experimental animals are infected with some facility, a localized lesion being produced at the site of subcutaneous inoculation in guinea pigs and rabbits, and generalized infection in dogs and rats.[129] Both yeast-like and mycelial forms are infectious experimentally.

Diagnosis.—Microscopic demonstration of the fungus is highly suggestive but culture and identification are required to establish the diagnosis. The disease has not been studied sufficiently to allow the develop-

[131] DeMonbreun: Amer. Jour. Trop. Med., 1939, *19*:565.

ment of a diagnostic procedure. *H. capsulatum* has been isolated by blood culture in generalized infections and from biopsy specimens; sternal puncture may prove useful. In any case, the mycelial form of the fungus developing on Sabouraud's agar at room temperature is distinguished from other pathogenic fungi by the formation of the large, thick-walled, tuberculate chlamydospores described above.

CHAPTER 33

THE SPIROCHETES

Spirally shaped microorganisms were for a long time grouped together indifferently under the common name of spirillium, spirochete or vibrio. Further knowledge has shown that some of these are true bacteria, although curved, and such forms are now placed with the bacteria in the genus *Vibrio* and various species of *Spirillum*. Others, however, possess characteristics which distinguish them from curved bacteria, such as the cholera vibrio, and also from the actively motile protozoa, such as the trypanosomes. These forms, the spirochetes, are actively motile but, unlike the motile bacteria, not by means of flagella but rather through the rotation of the screw-shaped cell. Some spirochetes possess a terminal filament which functions as an organ of locomotion and resembles the polar flagellum of monotrichous bacteria. Like the bacteria, however, the spirochetes multiply by transverse fission, have no well-defined nucleus, and do not exhibit anterior-posterior polarity. They appear to be more closely related to the true bacteria than to the protozoa and may, perhaps, be regarded as a connecting link between the two.

Classification and nomenclature within the group are still in an uncertain condition, and there is by no means general agreement regarding the separation of some of the genera. These microorganisms are difficult to cultivate on artificial media in many cases, and little or nothing is known of their physiological characteristics; the genera are separated from one another on a purely morphological basis.[1]

Although the name spirochete is commonly used to designate those forms which are classified as members of the family *Spirochaetaceae*, the generic term *Spirochaeta* was originally proposed by Ehrenberg[2] for the large free-living forms he described. A second genus, *Cristispira*, is characterized by the presence of a membranous appendage or crista wound about the body of the cell, and is made up of microorganisms found living as saprophytic commensals in certain molluscs. A third genus, *Saprospira*, consists of free-living forms which differ from *Cristispira* in that the crista is absent. The other genera contain the spirochetes which are pathogenic for man and animals: *Borrelia*, the spirochetes of the relapsing fevers; *Leptospira*, the spirochetes causing Weil's disease or infectious jaundice and certain fevers; and *Treponema*, the spirochetes of syphilis and yaws. The differential characteristics of these genera are summarized in the accompanying table.

[1] *Cf.* the detailed discussion of Noguchi: *The Spirochetes*. Chapter XXXVI in Jordan and Falk: *Newer Knowledge of Bacteriology and Immunology*. The University of Chicago Press. Chicago. 1928.

[2] Ehrenberg: *Die Infusionstierchen*. 1838.

CLASSIFICATION OF SPIRAL MICROORGANISMS

		Spirochaeta ("Coil-hair")	Saprospira ("Putrid-coil")	Cristispira ("Crested coil")	Borrelia (Borrel: Compt. rend. Soc. biol., 1906, *60*:138)	Treponema ("To Turn, Thread")	Leptospira ("Fine-coil")
Measurements in microns	Length......	100 to 150	100 to 120	45 to 90	8 to 16	6 to 14	7 to 9 or 14
	Diameter....	0.5 to 0.7	....	1.0 to 1.5	0.35 to 0.5	0.25 to 0.3 Cylindrical	0.25 to 0.3
	Width of spiral......	2	....	....	....	1.0 Regular, rigid	0.45 to 0.5
	Length of spiral......	1.5 Regular	....	....	....	0.8 to 1.0 Very constant	0.3 Regular
Shape of ends......		Blunt	....	Blunt	Pointed	Pointed	Pointed
Waves (in addition to finer spirals).		Several large, inconstant, irregular.	Large, inconstant, shallow, irregular, 3 to 5 in number.	Two to five or more. Large, irregular, shallow.	Large, wavy spirals, usually five.	One or more slight undulating waves.	One or more gentle wavy curves throughout entire length. One or both ends semicircularly hooked when in liquid media. Serpentine in semisolid media. Extremely flexible.
Axial filaments.....		Present (flexible elastic)	Absent	Absent	Present	Present	Absent
Chambered structure		Absent	Present	Present	Absent	Absent	Absent

Membrane.........	Absent	Present (flexible elastic)	Present (flexible elastic)	Delicate, flexible, double contoured.	Doubtful	Absent
Terminal (finely spiral filament)......	Absent	Absent	Absent	Present	Present	Not recognized
End (highly motile end portion).	Absent	Absent	Absent	Absent	Absent	Well developed in last 6–8 spirals.
Crista (undulating membrane).	Absent	Absent	Present; ridge-like membrane spirally about body.	Absent	Absent	Absent
Division (character).	Transverse	Transverse	Transverse	Transverse, possibly also longitudinal.	Transverse, possibly also longitudinal.	Transverse
Habitat...........	Fresh or marine water.	Foraminiferous sand.	Parasitic in alimentary canals of shell fish.	Numerous pathogenic and non-pathogenic varieties.	Two pathogenic and several harmless parasites.	Two pathogenic varieties and one possibly non-pathogenic.
Action of chemicals: Trypsin digestion.	Axial filament resistant.	....	Membrane resistant. Crista and chambers disappear.	....	Resists for many days.	
Bile salts (10 per cent).	Becomes shadowy, pale, but is not dissolved.	....	Crista destroyed. Body not attacked.	Complete disintegration.	Complete disintegration.	Easily dissolved.
Saponin (10 per cent).	Lives thirty minutes; later becomes shadowy, pale, but not dissolved.	....	Crista becomes fibrillar and then indistinct. Body not affected.	Immobilized in thirty minutes. Broken up in a few hours.	Eventually broken up.	Completely resistant.

THE SPIROCHETES OF THE RELAPSING FEVERS (BORRELIA)[3]

The relapsing fevers are a group of closely related infections characterized clinically by an initial pyrexia of three to four days' duration, followed at intervals of a few days by successive relapses. They are widely distributed and occur in every country of the world with the possible exception of Australia. The microorganisms responsible for these diseases are spiral forms, first observed by Obermeier[4] in the blood of patients with European relapsing fever.

Morphology.—The basic structure of these spirochetes is a springlike axial filament upon which there is a layer of contractile protoplasm enveloped in a delicate periplast. The terminal filaments are, perhaps, drawn-out ends of the periplast or non-rigid and non-coiled ends of the axial filament. Attached spherical bodies are sometimes observed and these are thought to be minute extrusions of cytoplasm through the periplast. The cell

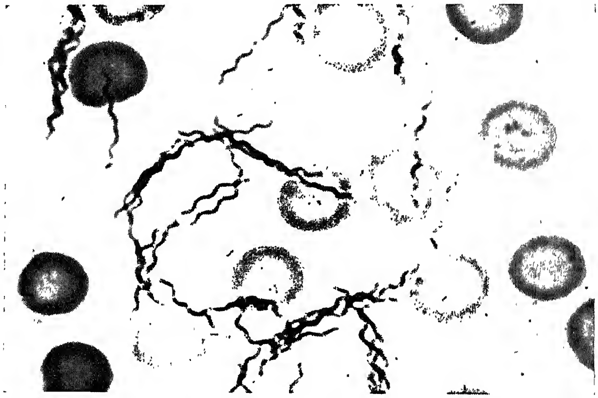


Fig. 183.—*Borrelia recurrentis* in blood smear. × 2000 (Kral).

rotates as a consequence of the stretching of the axial filament by the pressure of contracting protoplasm. When there are rapid and successive contractions and relaxations, the microorganism rotates rapidly and moves in one direction or another according to the direction of the rotation. In the relaxed state the spirals are regular and even; the larger the cell the heavier the axial filament and the greater the distance between the spirals. The regularity of the spirals is disturbed by the contracting and relaxing protoplasm in the actively motile cells. The various species cannot be distinguished from one another on a morphological basis. All are 0.2 to 0.5 μ in breadth by 10 to 20 μ in length. They are best stained by the Romanowsky method or some modification such as Giemsa, but, in contrast to some of the others, *Borrelia* may be stained with the ordinary aniline dyes.

[3] For general discussions see A.A.A.S. Symposium No. 18, *Relapsing Fever in the Americas*, 1942; Hemingway, Hemingway and Arneson: Northwest Med., 1940, *39*:362.

[4] Obermeier: Centralbl. f. d. med. Wissenschr., 1873, *11*:145.

Cultivation.—The spirochetes of relapsing fever have been cultivated by Noguchi[5] in a medium of ascitic fluid and fresh tissue in which a network of fibrin is formed. A semisolid serum agar has been used by Kligler and Robertson.[6] In culture the spirochetes are aerobic and the paraffin-oil seal frequently used prevents evaporation rather than interferes with the diffusion of oxygen. While such successful cultivation has been reported, the spirochetes are exceedingly difficult to grow in initial culture and cannot be maintained in serial passage; it is not unlikely that they only persist for a time in such media and never have been actually cultivated. They may be grown in the developing chick embryo,[7] however, and have been isolated directly from human infections in this way.[8]

Classification.—The relapsing-fever spirochetes have been given the generic name *Borrelia* and are so called in the Bergey classification. The validity of this degree of differentiation from the spirochetes of syphilis

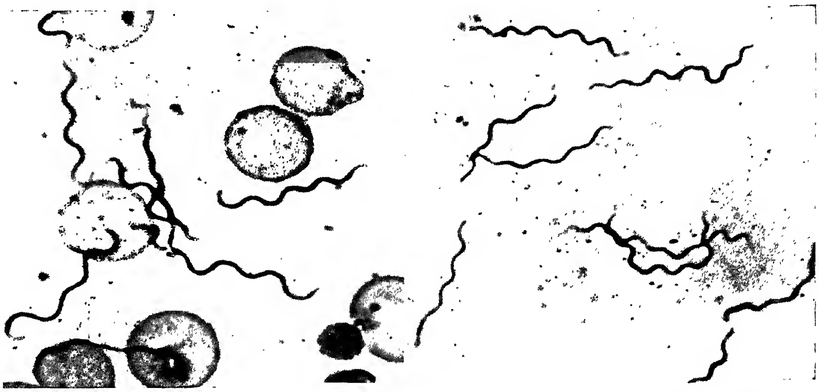


Fig. 184.—Relapsing fever spirochetes. Left, *Borrelia duttonii* of Central African relapsing fever; right, *Borrelia kochii* of East African relapsing fever. × 2000 (Kral).

and yaws, *Treponema*, is open to some question, and the relapsing-fever spirochetes are regarded by many as *Treponema* species. It has been pointed out by Noguchi[1] that the relapsing-fever spirochetes do not differ more from *Treponema pertenue* (yaws) than that microorganism differs from *Treponema pallidum.*

It is not clear to what extent the relapsing fever spirochetes can be divided into species. It is probable that in many cases those given species rank are, in fact, only strains or varieties. In many instances these have been given place names or named after some individual worker but the differentiation has been geographical rather than biological. These forms are immunologically heterogeneous and unstable in that they are modified in character by residence in different insect or mammalian hosts.

[5] Noguchi: Jour. Exp. Med., 1912, *16:*199, 620.
[6] Kligler and Robertson: Jour. Exp. Med., 1922, *35:*303.
[7] Chabaud: Bull. Soc. Path. Exot., 1939, *32:*483; Oag: Jour. Path. Bact., 1940, *51:*127
[8] Bohls, Irons and DeShazo: Proc. Soc. Exp. Biol. Med., 1940, *45:*375.

Cross-immunity tests, therefore, are not useful. It has been suggested that they be differentiated on the basis of pathogenicity or insect host but there appear to be no reasonably definite lines of demarçation possible on these bases. In general, then, the questions of interrelationship and speciation of these organisms are almost completely open at present, but most workers agree that, although they are all immunologically related, there is too great heterogeneity to justify regarding the various strains as no more than varieties of a single species *Borrelia* (*Treponema*) *recurrentis*.

Pathogenicity.—As indicated above, the relapsing fevers constitute a group of closely related diseases. The spirochete observed by Obermeier was that of European relapsing fever, a disease which has been known since the early part of the eighteenth century and has at times prevailed extensively in parts of Europe. The causal microorganism is designated *Borrelia* (or *Treponema*) *recurrentis*. Four forms of relapsing fever occur in Africa; the spirochete of the form in Central Africa is *Borrelia duttonii*, that of the East African disease *Borrelia kochii*, that occurring in northern Africa, in Tunis, Algiers and Tripoli, is *Borrelia berbera*, and that in the Sudan *Borrelia aegyptica*. Relapsing fever also occurs in India, and the causative microorganism is *Borrelia carteri*. American relapsing fever is caused by still another spirochete, *Borrelia novyi*; other strains isolated in this country have been named *Borrelia turicata*. South American strains have been called *Borrelia venezuelensis* and *Borrelia neotropicalis*.

All forms of relapsing fever are clinically identical. The onset is sudden, with chills, fever and severe headache, muscular and joint pains are frequently observed, and there is a moderate enlargement and tenderness of the spleen. The fever ends suddenly by crisis in three or four days. Successive relapses occur at intervals of two to fourteen days, and the period of relapse varies from a few hours to longer than the primary fever. The number of relapses varies. Spirochetes may be found in the blood during the paroxysms. The case fatality is not high in European relapsing fever, perhaps 4 to 5 per cent. There are no characteristic findings at autopsy.

The infection may be transmitted to the rat and mouse, but the guinea pig appears resistant to most strains though it may be infected with certain American strains.[9] It has been reported that the hamster may be infected with at least some strains.[10] It is of some importance that the blood for inoculation be taken at the onset rather than at the decline of the relapse. Ordinarily the infection is inapparent in both rats and mice in that there are no observable symptoms, but successive "attacks," beginning two to four days after inoculation, are evidenced by the appearance of the spirochetes in the blood. They are present for two or three days, disappear, and reappear three or four days later in a second "attack." Usually only two or three such relapses occur, the spirochetes becoming fewer and persisting for shorter times in the successive relapses. Experimental dis-

[9] Davis: Pub. Health Repts., 1939, *54*:1721.
[10] Chen and Anderson: Proc. Soc. Exp. Biol. Med., 1941, *46*:658.

ease may also be produced in monkeys and its course is very similar to that in man.[11]

Natural infection of lower animals occurs with some frequency in endemic areas and there is much evidence to support the hypothesis of Nicolle and Anderson[12] that small mammals, especially rodents, constitute a natural reservoir of the tick-borne infection. A wide variety of animals may be infected, including the rat, mouse, chipmunk, squirrel, opossum, porcupine, armadillo, hedgehog, fox and dog. Susceptibility varies considerably among animal species, and from one strain to another of the spirochete.

A spirochetosis of geese is due to *Borrelia anserinum*, a microorganism regarded by many as identical with *Borrelia gallinarum*, which produces a septicemia of chickens. Another species, *Borrelia thieleri*, is associated with a benign affection of cattle in South Africa but appears to have but low pathogenic powers. Morphologically similar spirochetes have also been found in the blood of sheep, horses and bats and in the alimentary tract of fish and insects, but they are not associated with any obvious indisposition.

Immunity.—Following infection agglutinins, lytic and spirocheticidal antibodies may be found in the serum. Usually the immunity following recovery from an attack of relapsing fever is of short duration but occasionally a more solid immunity may develop. The latter is often attributed to a persistence of the infection *i.e.*, an immunity to superinfection.[13] This is not definitely known, however.

Relapse appears to be basically an immunological phenomenon and dependent upon the antigenic instability of the spirochete. It was shown by Novy and Knapp[11] that the spirochetes will remain alive for as long as forty days in blood drawn before the onset of an attack, but in blood drawn during the decline of an attack or after recovery they die out in less than an hour. In the latter case the blood is spirocheticidal and the killed spirochetes are phagocytosed. It was suggested by Novy[14] that the relapse is a consequence of the survival of a few individuals which are resistant to the specific spirocheticidin and which multiply to give rise to a new serum-fast strain. It has been found by Cunningham and his coworkers[15] and by Meleney[16] that spirochetes isolated after successive relapses differ serologically from the spirochetes of the first attack, and as many as nine different serological types of *Borrelia duttonii* were distinguished by Cunningham; after any particular type appeared in an animal, it never reappeared in the further course of the disease in the same animal. It is not clear whether this immunological variation is "normal" in the sense that it

[11] Experimental infections have been studied in detail by Novy and Knapp: Jour. Inf. Dis., 1906, *3*:291.

[12] Nicolle and Anderson: Bull. Inst. Pasteur, 1927, *25*:657.

[13] *Cf.* Hindle: Trop. Dis. Bull., 1935, *32*:309; Coleman: Jour. Inf. Dis., 1934, *54*:1; Beck: Jour. Inf. Dis., 1937, *60*:64.

[14] Novy: Science, 1908, *27*:650.

[15] Cunningham: Trans. Soc. Trop. Med. Hyg., 1925, *19*:11; Cunningham *et al.*: Indian Jour. Med. Res., 1934, *22*:105, 595.

[16] Meleney: Jour. Exp. Med., 1928, *48*:65.

occurs without environmental stimulus as in the case of the phase variation of *Salmonella* (p. 387), the antiserum selecting out the resistant immunological type, or whether it is the kind of induced variation produced by cultivation of a microorganism in the presence of homologous antibody to give rough variants.

The parallel between this and the successive relapses of certain types of trypanosomiasis is striking. In the guinea pig infected with *Trypanosoma rhodesiense* recovery is due to the development of a specific trypanolysin, the relapse to the multiplication of serum-fast trypanosomes, second recovery to a second specific trypanolysin and so on. In this case the serum-fast strains are immunologically distinct from one another. In *Trypanosoma lewisi* infections in the rat, however, both a trypanolysin and a reproduction-inhibiting antibody, or ablastin, are formed; the inhibition of cell division does not, presumably, allow the development of serum-fast strains, and hence there are no relapses.[17]

Diagnosis.[18]—The diagnosis of relapsing fever is made by demonstration of the spirochetes in the blood during the onset of a relapse by direct microscopic examination or by animal inoculation. Either the usual blood smear or a thick film similar to that used for the detection of malarial parasites may be used. The films are air-dried and stained with Giemsa; Wright's stain is satisfactory for thin films. The spirochetes may also be found in fresh wet preparations by darkfield examination. Mice and/or rats may be inoculated intraperitoneally and blood smears made daily for as long as two weeks if the spirochetes are not found.

In the past immunological methods of diagnosis have not been satisfactory. More recently, however, Stein[19] has prepared spirochete antigens by saponin hemolysis and washing of the spirochetes. Complement fixation, said to be preferable for diagnostic purposes, and agglutination with sera from infected persons or animals occur and are reported to be specific.

Epidemiology.—The relapsing fever spirochetes are blood rather than tissue parasites and the infection is transmitted by blood-sucking insects, though rare cases of direct transmission may possibly occur. Two epidemiological types of the disease are to be distinguished, the one tick-borne and representing transmission from an animal reservoir of infection to man, and the other louse-borne infection spread from man to man.

A tick vector was not recognized in this country until 1930[20] but the tick-borne disease is now known to be not uncommon and at the present time is the only type which occurs. Known endemic foci of infection exist in Arizona, California, Colorado, Idaho, Kansas, New Mexico, Nevada, Oklahoma, Oregon, and Texas and possibly also in Montana, Utah and Washington. *Ornithodorus turicata* and *O. hermsi* are known to transmit the disease and it seems probable that *O. parkeri* transmits it also but final proof is lacking. *O. talaje* transmits the infection in tropical America but apparently not in the United States; *O. rudis* is regarded as the most

[17] *Cf.* Taliaferro: Jour. Exp. Med., 1924, *39*:171.
[18] *Cf.* Bohls and Schuhardt: Texas State Jour. Med., 1933, *29*:199.
[19] Stein: Jour. Exp. Med., 1944, *79*:115.
[20] Weller and Graham: Jour. Amer. Med. Assn., 1930, *95*:1834.

important vector in the tropics in this hemisphere. The traditional vector, and the most common in West Africa, is *O. moubata*. There appears to be no specific relationship between any particular species of tick and any particular strain of spirochete.

Infection may persist for long periods of time in the tick; Francis[21] has reported survival in starved ticks for as long as five years, and in refed ticks for six and one half years. Furthermore, the spirochete may be transmitted to the offspring and even the third generation may be infected. The spirochetes are present in the coxal fluid, saliva and feces of infected ticks. Of these, the first two appear to be the more important in transmission of the infection to man. For example, *O. moubata*, an excellent vector,

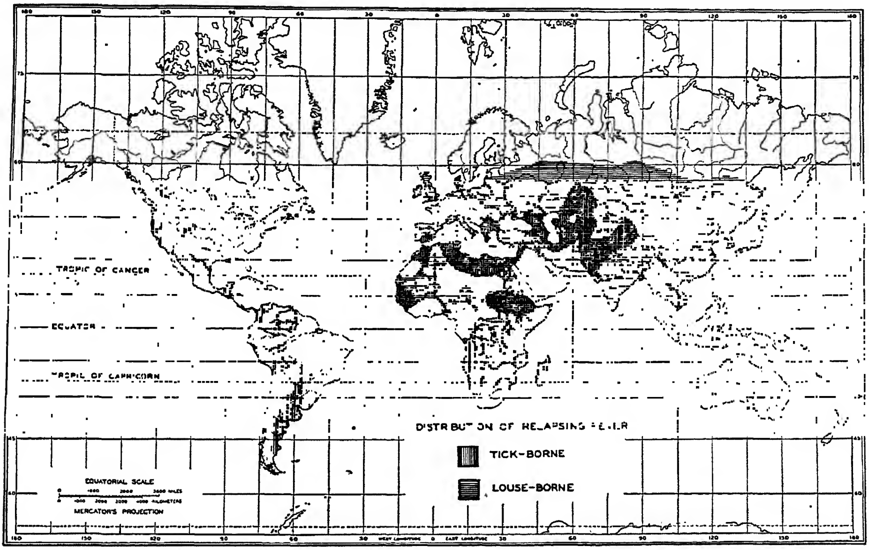


Fig. 185.—The world-wide distribution of relapsing fever, both louse- and tick-borne. Redrawn from map prepared by Army Medical Intelligence, 1943. Based on Goode *Base Map* No. 201M. By permission of the University of Chicago Press.

secretes coxal fluid copiously while feeding, thus making possible infection of the bite, while *O. hermsi*, which is not known to transmit the infection to man though it does so from rat to rat, does not pass coxal fluid while feeding. The relative importance of direct introduction of the spirochete into the bite by infected saliva is not altogether clear. It is not improbable that infection of the bite by contaminated feces occurs occasionally.

European relapsing fever is transmitted from man to man by the human body louse, *Pediculus vestimenti*, and has the epidemiological characteristics of louse-borne disease. In contrast to the tick-borne infection, the bite is not infected by the secretions of the lice; rather the infected louse must be crushed on the skin and the spirochetes present in the body fluids

[21] Francis: Pub. Health Repts., 1938, *53*:2220.

contaminate the bite. The infection is transmitted to the egg, and there is some evidence that the disease may be produced by crushed eggs laid by infected lice.

It is possible that the fingers may become contaminated by crushing infected lice and transmit the infection by scratching. The bedbug may also serve as a mechanical carrier of the infection.

Spirochetosis of fowls is transmitted by a tick, *Argas persicus*, which infests fowls in the warmer parts of the world and, as in human relapsing fever, the spirochete is transmitted through the egg of the tick to the offspring.

VINCENT'S ANGINA (BORRELIA VINCENTI)[22]

Vincent's angina, an ulceromembranous angina and stomatitis of the mucous membranes of the mouth and throat, is variously known as Plaut-Vincent's angina, pseudomembranous angina, trench mouth, etc. The symptoms and lesions are quite characteristic, and the affection is prevalent, though it generally passes unnoticed because of its mild character.

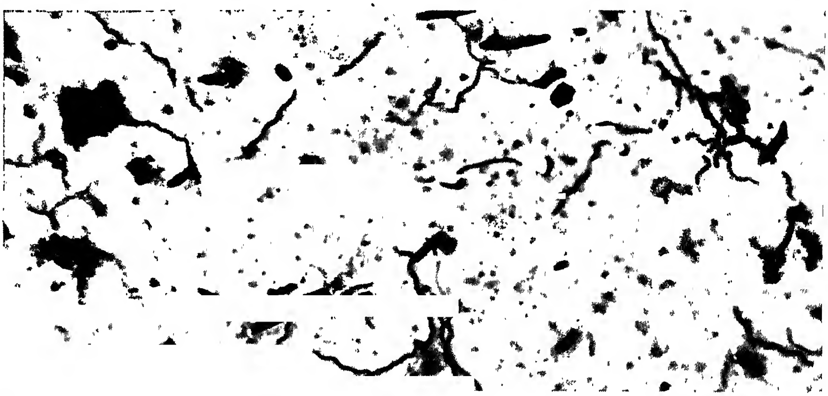


Fig. 186.—Smear from Vincent's angina showing the characteristic mixture of spirochetes (*Borrelia vincenti*) and fusiform bacilli. Fuchsin; × 1250 (P. E. Harrison).

Smears from the necrotic lesions show two types of bacteria intermingled, fusiform bacilli (p. 490) and spirochetes. The latter are morphologically similar to the relapsing-fever spirochetes and have been designated *Borrelia vincenti*. The relation of the spirochetes to the fusiform bacilli is by no means clear. It has been supposed by some that the two are distinct microorganisms, closely associated in some sort of symbiotic relation. Others[23] are of the opinion that these two forms are different phases in the life history of a single microorganism. The balance of the evidence is, however, against the latter view. The fusiform bacilli may be cultivated as indicated in an earlier chapter (Chap. 26), but as yet the spirochetes have not been grown on artificial media.

[22] See the review by Weaver: Med. Res. Council (Great Britain) Monthly Bull., 1943, *2*:107.

[23] Tunnicliff: Jour. Inf. Dis., 1906, *3*:481.

The causal relation of these microorganisms to Vincent's angina is not established. They are found in practically every normal mouth and in a variety of pathological conditions where their presence suggests that of a secondary invader rather than that of a primary exciting cause. Pure cultures of the bacilli injected into traumatized tissues have failed to produce lesions.[24] It has been found that dogs injected with a squill glucoside (scillaren B) develop a condition of the mouth identical with that of Vincent's angina. The etiology of this disease is as yet entirely unknown, as is the pathogenicity of the spirochetes of the mouth.[25]

SYPHILIS (TREPONEMA PALLIDUM)

Syphilis was not recognized as a clinical entity prior to 1493. The origin of the disease has been a matter of dispute, some maintaining that it was taken to Europe from the New World by the crew of Columbus, and others that it was already present in Europe.[26] In any case, some of the returning crew accompanied Charles VIII of France in the invasion of Italy in 1494. The epidemic which began in Italy at this time spread over Europe with the scattering of the troops. The sexual transmission of the disease was not realized until some time after this epidemic outbreak. The French referred to it as the Italian disease, and the Italians termed it the French disease or *morbus gallicus*. The disease derived its name from the principal character of the poem "*Syphilis sive Morbus Gallicus*" written by Fracastorius in 1530.[27]

The disease was transmitted to monkeys and anthropoid apes by Metchnikoff and Roux[28] in 1903, and the spirochete was described by Schaudinn and Hoffmann[29] in 1905.

Morphology.—As indicated earlier, the morphological differences between *Borrelia* and *Treponema* are minor, and *Treponema pallidum* need

[24] Lichtenberg, Werner and Lueck: Jour. Amer. Med. Assn., 1933, *100:*707.

[25] For discussions see Hanell: Arch. Otolaryng., 1931, *14:*1; Smith: *Oral Spirochetes and Related Organisms in Fuso-Spirochetal Diseases.* Williams & Wilkins Company. Baltimore. 1932; Gins: Ztschr. f. Hyg. u. Infektionskr., 1942, *124:*460.

[26] See Holcolm: Bull. Hist. Med., 1941, *10:*148.

[27] "The other poem, also by Fracastor, entitled *Siphilis*, dedicated to the future cardinal, Pietro Bembo,—the same who shunned reading the Epistles of Paul the Apostle lest he corrupt his own style,—hymned forth in line just as irreproachably elegant as the first, and also after Virgil, the French Malaise and the modes of treatment by sulphur baths and mercury ointment. The origin of the disease was explained, *in passim*, by the fable of a certain shepherd of ancient times by the name of Siphilis, who had aroused the ire of the god of the Sun with his mockeries; the god did punish him with a malady which would not yield to any treatment, until the nymph America did initiate him into her mysteries and did lead him to a grove of the healing Guaiacum trees, a sulphur spring, and a lake of mercury. Subsequently Spanish travelers, having sailed across the ocean and discovered the New Lands, wherein the nymph America dwelt, did also offend the god of the Sun, having shot in the chase the birds sacred to him, of which one did prophesy in a human voice that for this sacrilege Apollo would send them the French Malaise." Merejkowski: *The Romance of Leonardo da Vinci.* Guerney translation, Modern Library.

[28] Metchnikoff and Roux: Ann. Inst. Pasteur, 1903, *17:*809; *ibid.*, 1904, *18:*1, 657; *ibid.*, 1905, *19:*673.

[29] Schaudinn and Hoffmann: Arb. a. d. k. Gesund., 1905, *22:*527; Deut. med. Wchnschr., 1905, *31:*711.

not be further described here. Involution forms and granular forms may be seen[30] and Levaditi[31] believes that granules are viable and infectious. This spirochete is exceedingly difficult to see in the unstained state by transmitted light and is also refractory to stains. It may be observed unstained in fresh preparations in the darkfield and in smears by transmitted light in india-ink relief stains. The spirochete may be stained in smears by Giemsa's eosin azure and in tissue sections by the silver-impregnation methods used in neurological work. Somewhat simpler methods of staining with Victoria blue or carbol-gentian violet are reported to be satisfactory.[32]

Cultivation.—Schereschewsky[33] reported the cultivation of *T. pallidum* on artificial media in 1909, but his cultures appear to have been contaminated with other microorganisms. Noguchi[34] later reported the growth of several strains of the spirochete in pure culture under strictly anaerobic conditions in a serum water medium containing a piece of sterile fresh rabbit kidney or testicle. These cultures were carried through

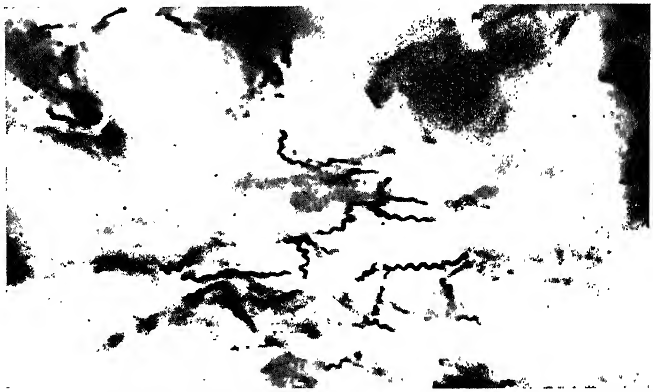


Fig. 187.—*Treponema pallidum* in stained (silver impregnation) smear. × 2000 (Kral).

a number of generations and produced typical lesions on intratesticular inoculation in the rabbit. The spirochete is very difficult to grow, especially in the first generation from the animal body. Noguchi first grew it from syphilitic lesions in the rabbit but was later able to cultivate it directly from man. Culture in the developing chick embryo is not successful.[35] Culture of the spirochete is, therefore, not a feasible routine procedure, and the microorganism is generally kept in the laboratory in infected animals. The spirochetes remain alive indefinitely in the lymphatics of the rabbit and may be readily obtained by excision of a popliteal gland.

[30] See the detailed morphological studies of Manouelian: Ann. Inst. Pasteur, 1940, *64*:439.

[31] Levaditi: C. R. Soc. Biol., 1941, *135*:467.

[32] Goldsworthy and Ward: Jour. Path. Bact., 1942, *54*:382.

[33] Schereschewsky: Deut. med. Wchnschr., 1909, *35*:835.

[34] Noguchi: Jour. Exp. Med., 1911, *14*:99.

[35] Callaway and Sharp: Jour. Lab. Clin. Med., 1941, *27*:232; Wile and Snow: Jour. Invest. Dermat., 1941, *4*:103.

It has been found[36] that the microorganism also remains viable indefinitely in the mouse and may be recovered from the lymphatics, spleen or brain. Levaditi[37] is of the opinion that different strains differ in their pathogenicity for experimental animals.

T. pallidum is a very fragile microorganism and dies out quickly outside the body. It is readily killed by drying and is unusually susceptible to heat; suspensions of infected rabbit testicle are sterilized by exposure to 41.5° C. for one hour and 41° C. for two hours.[38] In undisturbed cultures kept uninterruptedly at 37° C. it may remain alive for as long as a year. Like other bacteria, this spirochete is much less affected by extreme cold. It remains viable for at least a year in infected tissue frozen at —78° C. and stored at that temperature; stored at —20° C., however, it dies out in forty-eight hours.[39] The risk of transmission of syphilis by blood from blood banks is negligible for the spirochete dies out in three days at refrigerator temperature.[40]

Pathogenicity for Man.—Syphilis is transmitted from person to person through intimate contact, usually sexual. Indirect transmission may occur, however, through the agency of articles such as common drinking cups, towels and the like, if the time interval is very short. The spirochete penetrates the skin and mucous membranes through minute abrasions and possibly through the intact skin, though this is not definitely known. An appreciable lapse of time is required for penetration, however, for it was early shown by Metchnikoff and Roux[28] that the application of calomel ointment one to two hours after cutaneous inoculation of chimpanzees prevented infection.

The prevalence of syphilis is not known definitely; the estimate that 10 per cent of adults in the United States will give positive Wassermann tests is probably fairly accurate. It is also estimated that between 1 and 2 per cent of the children in this country have congenital syphilis. The mortality from syphilis is also not known. The death rate of 13 to 14 per 100,000 is misleading, for deaths reported as due to other causes are frequently due to syphilis; all cases of general paresis, locomotor ataxia and many cases of apoplexy and aortic aneurysm are, for example, due to syphilitic infection, and the disease is a contributory cause in deaths from other diseases. Studies on the incidence of syphilis in autopsies on adults have given rates from 2.6 per cent to 29.5 per cent; the average in 146,761 autopsies performed from 1896 to 1938 was 5.45 per cent.[41]

In this country the prevalence is much higher in the Negro than in the white; tests of the first two million selectees under the Selective Service Act showed 2.3 per cent in white and 27.2 per cent in Negroes. There is considerable variation in the ratio from one part of the country to another.[42]

[36] Kolle and Schloszberger: Deut. med. Wchnschr., 1928, *54:*129.
[37] Levaditi: Ann. Inst. Pasteur, 1942, *68:*118.
[38] Boak, Carpenter and Warren: Jour. Exp. Med., 1932, *56:*725.
[39] Ravitch and Chambers: Bull. Johns Hopkins Hosp., 1942, *71:*299.
[40] See the discussion in the Lancet, 1941, *i:*286.
[41] These studies have been summarized by Rosahn and Black-Schaffer: Arch. Int. Med., 1943, *72:*78.
[42] See Vonderlehr and Usilton: Jour. Amer. Med. Assn., 1942, *120:*1369.

Smillie,[43] therefore, regards syphilis in the United States as primarily a Negro problem.

A disease of protean manifestations, syphilis in man assumes several relatively well-defined clinical stages. The ordinary course of the disease includes the successive appearance of the so-called primary, secondary and tertiary stages; congenital syphilis is best regarded as a separate subdivision.

Primary Stage.—Following the penetration of the skin or mucous membranes, the spirochetes rapidly invade the tissues; it has been found that the inguinal lymphatics of rabbits become infective thirty minutes after cutaneous inoculation of the scrotum.[44] The initial primary lesion appears ten to thirty days following infection. The typical hunterian chancre (so called because it was described accurately and in detail by John Hunter) is an indolent, indurated ulcer, single and painless, and not infrequently atypical and trifling in appearance. The presence of the spirochetes in the chancre serum may be demonstrated by darkfield examination and constitutes the diagnostic method in early primary syphilis, for a positive Wassermann reaction (see below) does not develop until two or three weeks after infection. Clinically, then, syphilis in the primary stage is a localized infection, but bacteriologically it is a generalized infection, and spirochetes are often present in the blood stream.

Secondary Stage.—In the secondary stage the generalized nature of the infection is apparent. Bones, joints, eyes and other organs are invaded with the appearance of constitutional symptoms, cutaneous lesions, enlargement of the lymph glands, pains in the joints and the like. Evidences of the development of this secondary stage are usually apparent four to eight weeks after the appearance of the chancre but may be delayed for a year or more. Spirochetes are present in all the lesions and may be found in the blood stream.

Tertiary Stage.—The disease passes insensibly into the tertiary stage and may persist for years. Ulcerating necrotic lesions of the skin and gummata of the internal organs are common, and the clinical manifestations of infection are so highly diverse that there is no characteristic picture. Spirochetes are present in the tertiary lesions but only in small numbers.

Neurosyphilis.—Spirochetal invasion of the central nervous system may occur independently of the so-called stages and as soon as the early secondary stage. Generally, however, neurosyphilis is a manifestation of late tertiary infection and may appear years after the initial infection, and tabes dorsalis, general paralysis and other evidences of the invasion are sometimes termed quaternary or parasyphilitic affections. The spirochetes may be found in the cord in tabes dorsalis and in syphilitic brains in the cortex, in the pia and vessel sheaths. General paralysis is, then, a diffuse spirochetosis of the brain involving the cortical layers chiefly, and the presence of the spirochetes in the parenchyma explains much of the histopathological picture and the nervous symptoms of the affection.

Latent Syphilis.—In some instances the primary lesion is not noticed

[43] Smillie: Jour. Amer. Med. Assn., 1943, *122*:365.

[44] Kolle and Evers: Deut. med. Wchnschr., 1926, *52*:1075.

and the secondary manifestations may be slight, and with their subsidence there is no clinical evidence of disease and the infection remains latent within the tissues. In other instances the latent condition develops after primary and secondary symptoms of usual severity. Such clinically latent infections may, perhaps, be regarded as the maintenance of a biological balance between the host and the parasite. Latent infections may, of course, develop clinical manifestations at a later time.

Congenital Syphilis.—The fetus is readily infected in *utero* by spirochetes passing through the placenta. The infection may result in a cessation of development and abortion, or the fetus may go to term and be born dead. In those who survive, the disease is generalized from the beginning, and the lesions are those of tertiary syphilis. Spirochetes may be demonstrated in great numbers in the tissues and organs affected. Third generation syphilis has been reported but is very rare.[45]

Immunity.—At the present time syphilis is not so severe a disease in man as it was in the early sixteenth century. Whether this is an expression

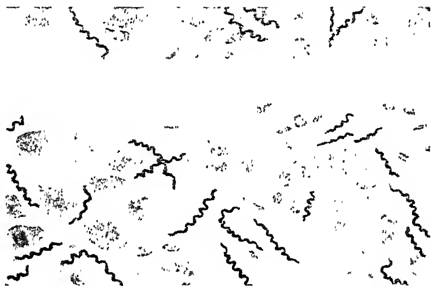


Fig. 188.—*Treponema pallidum* in congenital syphilis; × 1000; Levaditi (Sobernheim, in Kolle and Wassermann).

of an adaptive response on the part of man with the development of a low degree of natural immunity or a similar response on the part of the parasite manifested as a decrease in virulence, is not known; possibly both may have occurred.

The specific immune response to syphilitic infection is not well understood. There is a marked apparent insusceptibility to reinfection that is illustrated by the fact that a second chancre may be produced by reinfection prior to the appearance of the first chancre, but after the first chancre has appeared further reinfection does not produce an initial lesion. It is commonly stated that man, once infected, is refractory to reinfection and that reinfection occurs only very rarely. In other words, there is an infection-immunity or immunity to superinfection superficially similar to that observed in tuberculosis. The refractory state may persist after "cure," but there is no assurance that the disease may be "cured" in the sense that the spirochete is completely eliminated from the tissues.

Evidence obtained in animal experiments suggests that this immunity may be more apparent than real. It has been shown, for example, that

[45] See summary of reported instances by Maijala: Acta Med. Scand., 1940, *103*:1.

spirochetes inoculated after the development of primary lesions gain access to the tissues even though they produce no new primary lesion. Whether a similar situation holds in man and the observed refractory state is only a "clinical immunity" or whether there is a genuine immune response is not clear.

Serodiagnosis.[46]—A type of antibody activity, attributed to the presence of a substance designated *reagin*, is developed as a consequence of infection with *T. pallidum* which has great practical value since it allows the serological diagnosis of syphilis. A complement fixation test was proposed by Wassermann and his co-workers[47] as an immunologically specific reaction, using a watery extract of syphilitic fetus as the antigen. It was soon found, however, that syphilitic sera fix complement in the presence of an extract of normal tissue.[48] Not long after the development of the Wassermann test it was observed that syphilitic sera produce a flocculation when mixed with Wassermann antigen. The reacting substance present in the antigen preparations occurs in various tissues but heart (beef) muscle extracts are the most satisfactory. It is soluble in alcohol and insoluble in acetone, and its activity is considerably increased by the addition of cholesterol.

Types of Tests.—Probably no serological reactions have been as intensively studied as the complement fixation and precipitation tests for syphilis, and the techniques have been modified and refined by many workers to increase sensitivity and specificity. At the present time two complement fixation tests, the Kolmer test and the Eagle complement fixation test, and four precipitation tests, the Kahn test, the Kline test, the Hinton test, and the Eagle precipitation test, are used.[49] Of these the most common are the Kolmer complement fixation and the Kahn precipitation tests. Detailed studies have shown that all these tests agree quite well, disagreement being most common with treated cases. In addition to these, more sensitive precipitation tests have been devised by Kahn and by Kline and are known as the Kahn presumptive test and the Kline exclusion test; they are apt to give false positive reactions but are regarded by some workers as having considerable utility as screening tests. Furthermore, the complement fixation test may also be quantitative, *i.e.*, the reagin present in the syphilitic serum may be titrated. For ordinary diagnosis this is unimportant since it is not known whether the reagin titer has any significance, but it is useful in following therapy and in the diagnosis of congenital syphilis in infants. In the latter case reagin is passively transferred from the maternal circulation; if the infant is not infected the reagin titer drops slowly, but a gradually increasing titer is indicative of infection. The usual qualitative complement fixation or precipitation test is used in the so-called provocative test in which a suspected latent

[46] For a detailed and critical discussion see *Modern Serologic Tests for Syphilis*. Ven. Dis. Inform. Suppl. No. 14, 1941.

[47] Wassermann *et al.*: Deut. med. Wchnschr., 1906, *32:*745; Ztschr. f. Hyg., 1907, *55:*451.

[48] Marie and Levaditi: Ann. Inst. Pasteur, 1907, *21:*138.

[49] Detailed instructions for the performance of these tests may be found in Ven. Dis. Inform., Suppl. No. 11, 1940.

syphilitic infection in a serologically negative individual is activated by a small dose of an arsenical, the test being run at intervals thereafter for ten days or more. All these tests may be, and frequently are, done with spinal fluid in cases of infection of the central nervous system.

The Effect of Therapy.—The majority of individuals undergoing therapy become serologically negative in time and this response is usually taken as an indication of the efficacy of the treatment. The time required to attain negativity is variable and depends upon the individual, the stage of the disease, the course of therapy, especially if continuous or intermittent, and the sensitivity of the serologic test used. No general statement of any precision is possible, therefore, but it is usually said that the majority of persons with primary or early secondary syphilis become negative after one or two courses of arsenical or continuous treatment for five to seven weeks. Some, however, become negative with only one or two injections of drug, while others may remain positive for months and a certain proportion remain positive indefinitely. The last is spoken of as Wassermann-fast, reagin-fast, or seroresistant syphilis. Slightly over 10 per cent of infections are in this group. Two explanations are usually offered for seroresistant syphilis, one that persistent foci of infection remain, and the other that the continued presence of reagin is evidence of a definite immune response.

Specificity and False Reactions.—It will be apparent that the serological tests for syphilis are not immunologically specific. Their specificity is, rather, an empirical one based on a high degree of association. Basically, therefore, aberrant reactions or departures from the association are not truly false, only false from the point of view of syphilology. They are, nevertheless, of considerable practical importance.

False negative reactions are in part a technical matter; serologic tests in general use give 80 to 90 per cent positive reactions with known sera and any test which gives more than 80 per cent positives is regarded as satisfactory. The presence of insufficient reagin accounts for many false negative reactions and occurs in several stages of the disease, especially in early primary syphilis (a positive reaction usually does not develop until two to three weeks after infection) and late syphilis which is latent or localized. Tabes, for example, gives about 40 per cent negative reactions.

False positive reactions may be a result of technical error or may be biological in nature. A certain proportion of non-syphilitic individuals suffering from other infections give positive serologic tests for syphilis. All cases of yaws are Wassermann-positive, of course, and it is reported that 4 to 10 per cent of cases of malaria are positive. Febrile disease of one sort or another occasionally produces isolated or repeated positive reactions. In general, however, false positive reactions do not occur with sufficient frequency to detract seriously from the diagnostic value of the serological tests.

The nature of the response to infection with the spirochete that results in the appearance of reagin is not clear. Its lack of immunological specificity would seem to rule out the usual type of antibody response, and if it is to be regarded as such, the facile disappearance of reagin during therapy rather than seroresistant syphilis requires explanation. It is possible, of

course, that reagin represents iso-antibody that is detectable only because it is being produced more rapidly than it can be removed by union with antigen. Phenomena that are possibly analogous have been observed in other diseases. Hughes[50] has shown that there is a precipitin present in convalescent yellow fever serum which reacts with serum taken during the acute stage of the disease but not with yellow fever virus; the precipitinogen has been regarded by some as a "pathologic protein." The observation of Avery, Abernathy and McLeod[51] is similar but reversed as regards specificity; they found that a substance appeared in the blood of human beings and monkeys during the course of various etiologically unrelated infections that specifically precipitated the somatic polysaccharide, or C substance, of pneumococci. In general, however, the diagnostic serologic tests for syphilis have contributed little to an understanding of immunity to this disease.

Pathogenicity for Lower Animals.—Syphilis may be transmitted to anthropoid apes, such as the chimpanzee and the gibbon, and, with less certainty, to monkeys. Scarification of the genitals or eyebrow results in the development of a primary chancre followed in a few weeks by the appearance of the lesions of secondary syphilis. Subcutaneous, intraperitoneal or intravenous inoculation is ineffective.

Rabbits may be infected by inoculation of the anterior chamber of the eye. The local wound heals, but in four to six weeks a pericorneal congestion develops which is followed by pannus and keratitis; then retrogression and healing take place. The entire process may take many weeks to complete. Intratesticular inoculation produces an orchitis and intrascrotal inoculation a primary chancre; generalized lesions characteristic of secondary syphilis follow. A generalized infection may be produced by the intravenous inoculation of very young rabbits.

In rats and mice no symptoms follow inoculation but the spirochetes multiply in the tissues and the infection remains latent indefinitely. Guinea pigs react in much the same way, though a local reaction may develop following intracutaneous inoculation in the perineal fold.

YAWS (TREPONEMA PERTENUE) AND BEJEL

Two treponemiases, closely related to and perhaps varieties of syphilis, may be differentiated from it and from one another on the basis of geographical distribution, method of transmission and certain clinical aspects of the disease. These are yaws or *frambesia tropica* and bejel or desert syphilis.

Yaws.—Yaws is a disease occurring in tropical countries and is common in Equatorial Africa, the tropical regions of the Far East though rare in India, the West Indies and tropical America. The causative agent is *Treponema pertenue* which was described by Castellani[52] in 1905.

The spirochete is found in the serous exudate of the cutaneous lesions and in the lymphatics. It may be demonstrated in Giemsa-stained smears

[50] Hughes: Jour. Immunol., 1933, *25*:275.
[51] Avery, Abernathy and McLeod: Jour. Exp. Med., 1941, *73*:173.
[52] Castellani: Brit. Med. Jour., 1905, *ii*:1280.

or by darkfield examination of fresh preparations, and is morphologically indistinguishable from *T. pallidum.*

The disease in man is characterized by a papular eruption. A general malaise precedes the appearance of the initial lesion which is practically always extragenital and takes the form of a single papule or a small group of them. The papule enlarges to a diameter of 3 to 4 mm., the thickened epidermis cracks and the fungoid mass beneath exudes a seropurulent fluid. The mass enlarges to 3 to 5 cm. in diameter and the lesion is termed a yaw. It eventially dries, leaving only a scar. Six weeks to three months after the primary lesion appears, a secondary eruption occurs which is similarly preceded by a general malaise. The lesions are of the same general

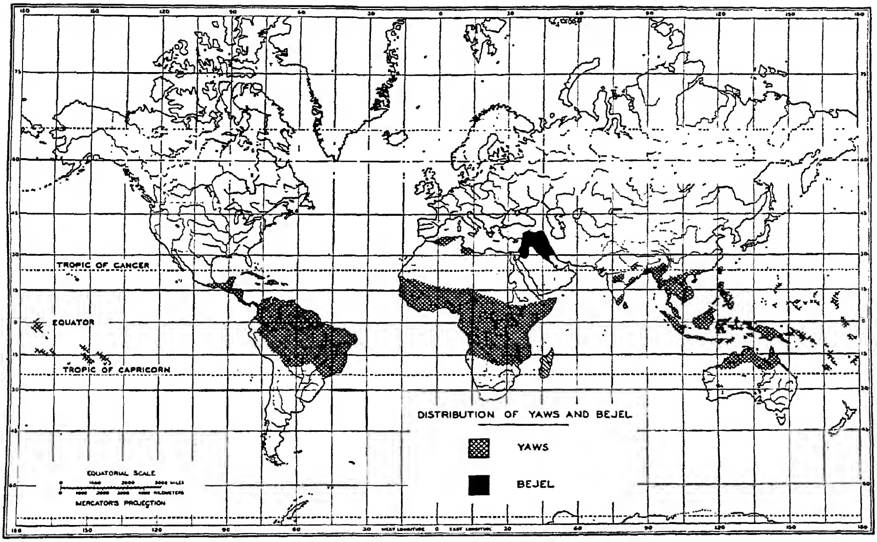


Fig. 189.—The world-wide distribution of yaws and bejel. Redrawn from map prepared by Army Medical Intelligence, 1943. Based on Goode *Base Map* No. 201M. By permission of the University of Chicago Press.

character as the primary lesion, appearing on the extremities, neck and at the juncture of the skin and mucous membrane about the nose, mouth and anus. Tertiary lesions similar to those of syphilis are said to be rare, but recent work suggests that they may be more common than has been supposed. The Wassermann reaction is positive and in certain stages, as in late secondary eruption, differential diagnosis may be very difficult.[53]

Yaws may be produced by the inoculation of monkeys and rabbits. The infection in rabbits with *T. pertenue* is similar to that with *T. pallidum* but relatively few spirochetes are found in the lesions, multiplication is arrested early, and the inflammatory reaction is slight.[54]

[53] *Cf.* Turner, Saunders and Johnston: Report of the Jamaica Yaws Commission, 1932.

[54] Ferris and Turner: Arch. Path., 1938, *26*:491.

The disease is not venereal and occurs predominantly in children. The spirochete is unable to penetrate the intact skin and enters via abrasions after deposition either by contact or by insects. Kumm[55] has found that in Jamaica the small fly, *Hippelates pallipes*, feeds upon the serous exudate in huge numbers, the spirochete surviving for some hours in the diverticulum of the fly and possibly being regurgitated when the fly feeds upon a skin abrasion.

The relationship of yaws to syphilis is very close, the points of difference being the non-venereal transmission of yaws, the differences between the yaw and the indurated hunterian chancre, and the relative infrequency of visceral and tertiary lesions. Yaws is regarded by many as a tropical type of syphilis, modified by continuous residence in the black races in tropical climates.[56]

Bejel.—A disease of desert Arabs, called by them bejel, has been reported by Hudson.[57] Like yaws it is non-venereal and occurs predominantly in children. It differs from yaws, however, in that mucous patches in the mouth occur with considerable frequency while they are uncommon in yaws. Nervous manifestations are said to be rare but Hoff and Shaby[58] have reported that meningovascular syphilis occurs with some frequency in Bedouin Arabs, if this may be called bejel. The disease has not been studied at length but appears to be very closely related to, if not identical with, syphilis, differing only in its non-venereal transmission. Butler[59] regards it as congenital syphilis. In this connection it is of interest that an outbreak of non-venereal syphilis in children and adult women, perhaps bejel, has been reported in India.[60]

PINTA (TREPONEMA HERREJONI)[61]

Pinta, known in Mexico as *mal de pinto* and in Colombia as *carate*, is clinically a skin disease in which dyschromic changes occur in patches which may be discrete and small or large and confluent, and of a grey, bluish grey, or pinkish color and eventually white. It is especially prevalent in Mexico where 11 per cent of over two million persons examined had the disease, and in Colombia where as much as 4 per cent of the population in some districts are affected. It also occurs in Cuba and the West Indian Islands, and in Central America and tropical South America. So far as is known it is confined to the Western Hemisphere.

Etiology.—For many years this disease has been of uncertain etiology though generally assumed to be a fungus infection. It was suggested by Herrejon in 1927 that the disease is caused by a spirochete but it was not until 1938 that a spirochete was first demonstrated by Armenteros

[55] Kumm: Report of the Jamaica Yaws Commission, 1935.

[56] See Chambers: Trans. Roy. Soc. Trop. Med. Hyg., 1937, *31*:245; Turner: Amer. Jour. Hyg., 1937, *25*:477.

[57] Hudson: Arch. Dermat. Syph., 1936, *33*:994; Amer. Jour. Trop. Med., 1938, *18*:675.

[58] Hoff and Shaby: Trans. Roy. Soc. Trop. Med. Hyg., 1940, *33*:549.

[59] Butler: Amer. Jour. Clin. Path., 1939, *9*:1.

[60] Iswariah and Nair: Jour. Indian Med. Assn., 1938, *7*:651.

[61] Recent literature has been summarized by Holcomb: U. S. Naval Med. Bull., 1942, *40*:517.

and Triana in Cuba. The observation has been amply confirmed and it is now established that pinta is caused by a spirochete which has been named *Treponema herrejoni* by the Cuban workers and *T. carateum* by Brumpt.

The spirochete may be found in material taken from early cutaneous lesions and in that aspirated from lymph glands. It stains with Giemsa and by the usual silver impregnation methods used for *T. pallidum*, and may be found by darkfield examination of fresh material. Morphologically it is very similar to, perhaps indistinguishable from, the spirochete of syphilis. Attempts to cultivate it have failed, but it has been recently reported that the rabbit may be infected. The experimental disease has been studied in considerable detail, however, especially by Leon y Blanco in human volunteers.

The Disease in Man.—In the experimental infection the incubation period is from seven to twenty days, the initial lesion being a papule which appears at the site of inoculation. This papule spreads peripherally to form a squamous, erythematous patch reaching a diameter of 1 cm. in four or five weeks. It continues to spread, varying considerably in appearance, and may be lichenoid or psoriaform. Blanco regards this as the primary stage. In about five months the secondary stage begins with the appearance of secondary lesions about the initial lesion and elsewhere on the body. Progressive hyperpigmentation occurs and depigmentation, resulting in varied hues, follows in the third stage; keratosis and superficial atrophoderma become apparent. Hyperkeratosis on the plantar surfaces occurs with some frequency in Cuba but is seldom observed in Mexico. The Wassermann reaction is almost always negative in the primary stage, positive in something over half the cases in the secondary stage, and practically always positive in the tertiary stage. Correspondingly, superinfection is readily produced in the first stage but not in the third stage. Syphilitic individuals may be infected without difficulty.

Transmission.—Experimental human infections have shown that serous fluid from the early lesion is highly infectious when placed on an abraded area on the skin, and the spirochete may be demonstrated in the discharge from the fissures in plantar hyperkeratosis. Contact infection would, therefore, appear to be possible but general opinion is that it is not an important method of spread. *Simulium* flies have been suspected of transmitting the disease, but as yet an insect vector has not been demonstrated.

OTHER TREPONEMAS

A number of other species of *Treponema* are known. *T. refringens* is found about the genitals and may be confused with the spirochete of syphilis. Others, *T. microdentium*, *T. macrodentium* and *T. mucosum*, are found in the mouth and apparently are non-pathogenic. A saprophytic water form, *T. elusum*, has been described.

Another species, *T. cuniculi*, is morphologically very similar to *T. pallidum* and is responsible for a venereal disease of rabbits known as rabbit syphilis and sometimes called pallidoidosis. The lesions are small, scaly patches found about the perineal region or on the genitals.

LEPTOSPIRA

(Weil's Disease or Infectious Jaundice; Swamp Fever; Japanese Seven-Day Fever)

Leptospirosis, or infection with leptospira, is a not uncommon and widely distributed affection. Several species of leptospira are involved and the infections differ somewhat in their clinical manifestations.

Morphology.—The leptospiras differ from *Treponema* and *Borrelia* species in that their spirals are very fine and close, one or both ends of the cell may be hooked, and the individual cells are smaller, not more than 0.3 μ in breadth and generally 6 to 10 μ in length, although exceptionally forms as long as 25 to 30 μ may be observed. The protoplasm is spirally wound about a delicate but firm, elastic, smooth axial filament. Extruding granules, found in *T. pallidum*, are not present even in electron micrographs.[62] Like the finer bacterial flagella, the axial filament is not seen in the darkfield or ordinary stained preparations but may be demonstrated by silver impregnation flagella stains. The periplast is thick and almost transparent and appears in the darkfield as a narrow, clear zone or halo and in certain stained preparations as a grayish or unstained halo. The mechanism of locomotion is more complex than in the treponemas; the hooked ends of the axial filament are probably involved, and when the bow-like axial filament is alternately straightened and relaxed by rhythmic contractions of the protoplasmic spirals the cell rotates.

The leptospiras are readily filterable through Berkefeld V and N candles because of their small breadth and active motility, and filtration is sometimes used to separate them from bacteria. Treponemas are usually not filterable by ordinary suction methods but grow through the pores of Berkfeld candles with some facility.

Cultivation.—The leptospiras are the most readily cultivable of the spirochetes and may be grown on serum diluted with 5 or 10 parts of Ringer or Locke solution. Growth is considerably better in a semisolid medium and Noguchi[1] has used a mixture of 1 part of 2 per cent nutrient agar, 1 part of rabbit serum and 8 parts of physiological salt solution. They have not been cultivated in synthetic media but Rosenfeld and Greene[63] have found that nicotinic acid and amide, thiamin, and riboflavin markedly stimulate growth. In the isolation of leptospira from water, agar medium and blood may be added to Berkefeld V and N filtrates.[64] According to Schüffner[65] the guinea pig may be used as a "living filter"; if leptospira contaminated with other bacteria are inoculated intraperitoneally, they enter the circulation much more rapidly than the contaminants and may be found in heart's blood in pure culture ten minutes after inoculation. The leptospiras are all obligate aerobes. Those tested do not ferment sugars and are indifferent to the presence of carbohydrates in the medium

Pathogenicity.—The spirochetes grouped in the genus *Leptospira* are immunologically related but distinguished from one another by aggluti

[62] Morton and Anderson: Jour. Bact., 1943, *45*:143.
[63] Rosenfeld and Greene: Jour. Bact., 1941, *42*:165.
[64] *Cf.* Bauer: Amer. Jour. Trop. Med., 1927, *7*:171.
[65] Schüffner: Centralbl. f. Bakt., I Abt., Orig., 1940, *145*:341.

THE LEPTOSPIROSES

Leptospira		Disease in Man	Animal Reservoirs		Infections in Other Animals	Geographical Distribution
Proper Name	Synonyms		Rats	Mice		
Lepl. icterohemorrhagiae.	"Wijnberg"	Weil's disease	*R. norvegicus* *R. rattus*	....	Dog, fox, cat, horse, cattle	World-wide, especially Europe and the Americas
Lepl. grippo-typhosa....	Andaman Sumatra 70, "Moskou V"	Swamp fever, Andaman fever	....	*Microtus arvalis,* *Apodemus sylvaticus* *Evotomys glareolus*	Dog, horse, cattle	Europe, N. E. I.
Lepl. sejroe...........	....	Swamp fever, infectious jaundice	"Rats" (Italy)	*Apodemus sulvaticus* *Mus spicilegus*	....	Denmark, Italy
Lepl. canicola..........	"Roesel"	Infectious jaundice	....	....	Stuttgart disease in dogs	World-wide
Lepl. hebdomadis.......	....	Japanese seven-day fever, nanukayami	....	*Microtus montebelli*	Dog	Japan
Lepl. autumnalis.......	Akiyami A, Rachmat	Hasami fever	"Field rat"	"Field mouse" *Apodemus speciosus*	....	Japan Sumatra
Lepl. bataviae..........	*Lepl. javanica*	Infectious jaundice	*R. norvegicus* *R. decumanus* (Japan) "Rats" (Italy)	*Apodemus sylvaticus* *Micromys minutus* *Micromys soricinus*	Cat	N. E. I., Japan, Italy
Lepl. pyrogenes........	Salinem	Infectious jaundice	*R. brevicaudatus* *R. rattus*	....	Cat	N. E. I.
Lepl. australis A.......	....	Mossman fever	*R. rattus* *R. culmorum*	....	....	Australia
Lepl. australis B.......	....	Mossman fever	*R. rattus* *R. culmorum*	....	....	Australia
Lepl. pomona..........	*Lepl. mezzana*	Pomona fever, swamp fever	"Rats" (Italy)	....	....	Australia, Italy

nation. Unlike *Borrelia*, they are not predominantly blood parasites but are also found in the tissues, especially the kidney, and are commonly excreted in the urine. The infections they produce in man are all similar and characterized by a febrile reaction and the occurrence of jaundice to a greater or lesser extent. Of these Weil's disease or infectious jaundice is perhaps the best known since it has occurred in Europe for many years. A related infection is found in Central and Eastern Europe and in Italy, and still others occur in the South Pacific region, in the East Indies and Australia, and also in Japan. These diseases have been reviewed by Walch-Sorgdrager[66] and are summarized in the accompanying table. The reservoir of infection is rodents, especially rats and various kinds of mice, and more recently the dog has been found to be of some importance. Human infection is probably almost always acquired from animals, usually by the contamination of water with infected urine.

Weil's Disease (Infectious Jaundice).[67]—The causative agent of Weil's disease, *Leptospira icterohemorrhagiae*, was discovered in 1914 by Inada and Ito[68] in Japan and was found in Germany the following year by Hübener and Reiter[69] and by Uhlenhuth and Fromme.[70] It was called *Spirochaeta icterogenes* by the German workers. The disease has been found all over the world; it is very common in Japan, less so in Europe where the greatest incidence is in the Netherlands and France, and is not uncommon in South America. It is probably not so rare in the United States as has been supposed.[71]

Disease in Man.—The incubation period is six to twelve days, and the high initial fever is followed by nausea and vomiting, epistaxis, headache and muscular pains, and there may be a moderately severe bronchitis. Jaundice is not always observed; it occurred in 40 to 60 per cent of the Dutch cases. Convalescence is slow and weakness may persist for months.

The leptospira are distributed throughout the body in the first week of illness and may be demonstrated in the blood by guinea-pig inoculation but rarely in blood smears. After the first week they are present in the urine and may continue to be so excreted for four to five weeks. There is some evidence that the case fatality is reduced by treatment with antiserum. In fatal cases death usually occurs during the second or third week, but occasionally as early as the end of the first week. The case fatality is variable; it has varied from 4.6 to 32 per cent in Japan and has been about 10 per cent in the Dutch cases and as high as 25 per cent in Scotland. At autopsy the leptospira are found in almost all the organs and tissues in those dying during the initial febrile stage, but if death occurs in the second week or later, they are rarely found elsewhere than in the kidneys.

Immunity.—Recovery from the disease is accompanied by the develop-

[66] Walch-Sorgdrager: Bull. Health Organization (League of Nations), 1939, *8:*143.

[67] The literature to 1941 has been reviewed by Ashe, Pratt-Thomas and Kumpe: Medicine, 1941, *20:*145.

[68] *Cf.* Inada *et al.:* Jour. Exp. Med., 1916, *23:*377.

[69] Hübener and Reiter: Deut. med. Woch., 1915, *41:*1275.

[70] Uhlenhuth and Fromme: Med. Klin., 1915, *11:*1202.

[71] See, for example, the experience recorded by Bruno, Wilen and Snavely: Jour. Amer. Med. Assn., 1943, *123:*519.

ment of a solid immunity, and a specific lysin is present in the blood which persists in detectable amounts for several years.[72] Agglutinins appear in the convalescent stage, sometimes to very high titer such as 1:100,000, but titers of 1:100 are significant. The agglutination test has considerable diagnostic utility in both man and animals.[73] Prophylactic inoculation has given encouraging results.

Transmission.—The wild rat is the most important animal reservoir of infection and Schüffner[74] has reported that 40 per cent of tame rats raised for experimental purposes have been found to be infected in Holland. The infection may be detected by darkfield examination of urine and kidney emulsion, and preferably by the inoculation of young guinea pigs with kidney emulsion. Larson[75] has found that a fatal infection may be produced in young mice, three to four weeks old.

The proportion of infected rats is variable from one locality to another; in New York about 4 per cent of the rats have been found to be infected, in Japan 40 per cent, in England 30 per cent, in Rotterdam 7 to 40 per cent and in Philadelphia about 10 per cent. The leptospira are discharged in the urine of infected rats and are transmitted to man via stagnant water contaminated with rat urine. Weil's disease is to some extent an occupational disease and occurs in coal miners, sewer workers and others working in contact with contaminated water.[76] In the first World War there were many cases in troops stationed in trenches containing stagnant water. The majority of the Dutch cases have been in swimmers, bargemen and fishermen and those who by accident or intent fall into the canals. In one instance a water-borne epidemic occurred in which the water was presumably contaminated by rats from a neighboring sewer.[77] The manner in which the spirochetes enter the body is not known, but very possibly it is through minute cuts and abrasions in the skin or via the alimentary tract.

Canine Leptospirosis.—The rôle of dogs in the transmission of infectious jaundice has been of considerable interest in recent years. Dogs suffer from two forms of leptospirosis, the one an acute jaundice ("yellows") similar to acute Weil's disease in man, and the other a non-jaundice type known as Stuttgart disease or canine typhus. *Lept. icterohemorrhagiae* may infect dogs and probably is responsible for the former type of disease, while a canine leptospira, *Lept. canicola,* produces the latter form. The relative incidence of the two infections in dogs is not known with certainty; some have suggested that as high as 50 per cent of canine leptospiroses are *icterhemorrhagiae* infections. *Lept. canicola* infection in dogs appears to be relatively common in the United States. Raven[78] found 38 per cent of rural dogs and 28 per cent of urban dogs examined in Pennsylvania

[72] See, for example, Packchanian and Nylah: Jour. Immunol., 1943, *46:*263.

[73] For the development of a macroscopic test see Starbuck and Ward: Jour. Inf. Dis., 1942, *70:*88.

[74] Schüffner: Ztschr. f. Immunitätsf., 1941, *99:*323.

[75] Larson: Pub. Health Repts., 1941, *56:*1546; *ibid.*, 1943, *58:*10.

[76] The epidemiology of leptospirosis is discussed by Molner and Kasper: Amer. Jour. Pub. Health, 1941, *31:*945.

[77] Jorge: Bull. Off. Int. Hyg. Pub., 1932, *24:*88.

[78] Raven: Jour. Inf. Dis., 1941, *69:*131.

showed serological evidence of infection; and Greene[79] found about 20 per cent of dogs selected at random and 29 per cent of dogs from hospitals in California showed the presence of antibody. Man may acquire Weil's disease from dogs and a number of human infections with *Lept. canicola* have been reported since 1934 in the Netherlands and more recently from this country.

Another species of leptospira, *Lept. sejroe*, has been described as the cause of human infection in Denmark[80] and in Italy. The cases showed milder symptoms than those infected with *Lept. icterohemorrhagiae*.

Swamp Fever.—A leptospiral infection prevalent during the summer and early autumn months in Bavaria, Silesia and in the Volga region is variously known as swamp fever, *Schlammfieber*, autumn fever, water fever, field fever, harvest fever, mud fever and slime fever. It has also been reported from Holland.[81] It is found among field workers following floods or in swampy districts particularly during the hay harvest. Clinically it closely resembles Weil's disease in a mild form—the case fatality is less than 1 per cent—but there is no jaundice except occasionally in the sclera. The causative microorganism, *Lept. grippo-typhosa* (*Spirochaeta grippo-typhosa*), is immunologically distinct from *Lept. icterohemorrhagiae*. The reservoir of infection is the field mouse, *Microtus arvalis;* the wood mouse, *Apodemus sylvaticus*, and the bank-vole, *Evotomys glareolus*, are less often infected. This disease has been of particular interest in recent years and has been reviewed by a number of workers.[82] A very similar disease is found in the rice fields in Italy but the etiology appears to be diverse in that a number of species of leptospira have been found, in many instances apparently identical with those found in the Far East.[83]

Leptospira Infections of the Far East.—A number of leptospira infections occur in the Far East similar to swamp fever in that they are febrile, jaundice is rare except in the sclera, and the case fatality rate is low. *Japanese seven-day fever*, or *nanukayami*, is prevalent in Japan in the autumn. The causative agent is *Lept. hebdomadis* and is carried by the field mouse, *Microtus montebelli*. A common sequal to the infection is opacification of the vitreous humor. *Hasami fever* is a similar disease also prevalent in Japan and caused by *Lept. autumnalis* (*Lept. Akiyami A*). The reservoir of infection is the mouse *Apodemus speciosus* and possibly other species of field mice and rats are infected.

Leptospirosis of heterogeneous etiology is not uncommon in the Netherlands East Indies. The disease occurring in Sumatra known as *Rachmat infection* is caused by *Lept. autumnalis* and is presumably identical with Hasami fever in Japan. Other infections are known by various names, including *Andaman A fever*, *Salinem infection* and the like. The causative organism of Andaman fever appears to be *Lept. grippo-typhosa*. The name

[79] Greene: Amer. Jour. Hyg., Sec. B, 1941, *34:*87.

[80] *Cf.* Petersen and Christensen: Compt. Rend. Soc. Biol., 1939, *130:*1507.

[81] Schuffner and Bohlander: Centralbl. f. Bakt., I Abt., Orig., 1942, *148:*264.

[82] Kathe: Med. Klin., 1941, *37:*892; Klin. Woch., 1942, *21:*791; Ztschr. f. Immunitätsf., 1943, *103:*60; Uhlenhuth: Ztschr. f. Immunitätsf., 1943, *103:*35; Schüffner: Klin. Woch., 1942, *21:*787.

[83] *Cf.* Mino: Muench. Med. Woch., 1941, *88:*96; Klin. Woch., 1942, *21:*337.

Lept. pyrogenes has been given the causal agent of Salinem fever. The remainder of the leptospiroses appear to be largely infections with *Lept. bataviae* which is found in natural infections in rats, including *Rattus norvegicus* and *R. decumanus* (in Japan), and in field mice including *Apodemus sylvaticus*, *Micromys minutus* and *M. soricinus*.

Mild fevers of leptospiral etiology are endemic in Queensland in Australia, and a number of immunologically distinct varieties of leptospira have been isolated. That designated *Lept. pomona* is the causative agent of the affection known as *Pomona fever*, and other species named *Lept. australis A* and *Lept. australis B* are responsible for febrile disease designated *Mossman fever*, *coastal fever* and the like. Additional varieties have also been reported but it is not as yet clear that they are distinct species.

Geographical separation of the leptospiroses is not altogether sound for at least some of these microorganisms occur in widely separated regions. Thus *Lept. grippo-typhosa* is found in the Netherlands East Indies and *Lept. bataviae* and *Lept. pomona* have been reported as the etiologic agents of swamp fever in Italy. There is as yet no evidence that leptospira other than *Lept. icterohemorrhagiae* and *Lept. canicola* occur in the United States.

Saprophytic Leptospira.—Leptospira closely resembling *Lept. icterohemorrhagiae* have been isolated from water by a number of workers. These non-pathogenic forms have been termed *Lept. biflexa* in this country and in England, and *Spirochaeta pseudoicterogenes* by the German workers. Their relation to the pathogenic leptospira is uncertain; Uhlenhuth and Zuelzer[84] have reported observations which suggested that the water leptospira are avirulent forms of *Lept. icterohemorrhagiae* whose virulence may be restored by animal passage. These results have not been confirmed by other workers.

RAT-BITE FEVER (SPIRILLUM MORSUS MURIS)

There are two distinct kinds of disease which may follow the bite of rats and are designated rat-bite fever. One, an actinomycete infection, is discussed elsewhere (p. 614). The other, known in Japan as *sodoku*, is caused by a spiral microorganism discovered by Futaki and his co-workers[85] in 1916. Following the bite of an infected rat, the original wound heals but, after an incubation period of ten to twenty-two days, becomes inflamed and painful. Fever, swelling of the lymph glands, skin eruptions and other symptoms occur. The fever is of the relapsing type, with paroxysms at fairly regular intervals, usually about once a week, which continue for one to three months or longer. The case fatality varies from 2 to 10 per cent.

The characteristic spiral microorganism has been found in the swollen local lesions of the skin and the enlarged lymph glands and also in two instances in the circulating blood. Guinea pigs and mice may be infected by the inoculation of blood or fluid expressed from the local lesions. The microorganism is found in about 3 per cent of the house rats in Japan. The rat is not the only vector of the infection, for cases of what is apparently

[84] Uhlenhuth and Zuelzer: Klin. Wchnschr., 1922, *1*:2124.

[85] Futaki, Takaki, Taniguchi and Osumi: Jour. Exp. Med., 1916, *23*:249.

the same disease have been traced to the bite of the cat, dog, pig, ferret, squirrel and weasel. Ripley and Van Sant[86] have reported the case of a medical student who acquired the infection from a cut during an operation on a dog.

The disease has long been known in Japan and has been reported from many localities all over the world. Brown and Nunemaker[87] have found a total of 125 cases reported in the United States from 1916 to the end of 1940. What proportion of the earlier reported cases were actinomycete infections is, of course, problematical. Of 40 cases reported in 1931–1940, the spirillum was demonstrated by animal inoculation in 17; of these 11 resulted from rat bite and the remainder had a history of mouse bite, cat bite or scratch, contact with dogs, or trauma without known animal contact. The natural occurrence of spirochetes in laboratory mice, rats and guinea pigs is a source of error to be guarded against in laboratory diagnosis by animal inoculation. There is little doubt that the affection is

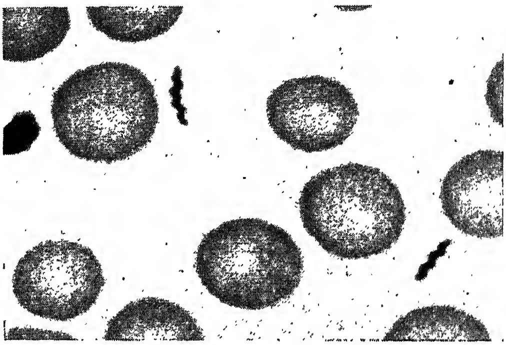


Fig. 190.—*Spirillum morsus muris* in blood of an inoculated mouse (Van Sant).

much more common than appears from the records, for the cases occur sporadically and their true nature has often gone unrecognized. The infection has been produced in man by artificial inoculation for the treatment of paresis; according to Brown and Nunemaker[87] there are 104 such cases in the English literature.

Not a great deal is known of the immune response to the infection other than that a spirillicidal antibody is produced that is also responsible for immobilization of the spirilla in immune serum. Savoor and Lewthwaite[88] have observed that a positive Weil-Felix reaction of the OXK type (p. 766) is produced in experimental animals but the antigen shared with these strains of *Proteus* is distinct from that which is responsible for the spirillicidal antibody.

The spiral microorganism of rat-bite fever has been variously classified as *Spirochaeta morsus muris, Borrelia muris, Spirillum minus*, etc. It is

[86] Ripley and Van Sant: Jour. Amer. Med. Assn., 1934, *102:*1917.
[87] Brown and Nunemaker: Bull. Johns Hopkins Hosp., 1942, *70:*201.
[88] Savoor and Lewthwaite: Brit. Jour. Exp. Path., 1941, *22:*274.

shorter—2 to 5 μ in length—than the recognized spirochetes, is relatively rigid and possesses polar tufts of flagella which give it a rapid darting motion unlike the flexible undulating movements of the spirochetes. The clinical symptoms of the disease are typical of spirochetosis, however, and the arsenicals are effective therapeutic agents. Originally given the name *Spirochaeta*, this microorganism was found[89] to be identical with *Spirillum minus* found by Carter[90] in the blood of a rat in India. It is, perhaps, best given the independent generic name *Spirillum*[91] and considered with the spirochetes.

[89] Robertson: Ann. Trop. Med. and Parasit., 1930, *24*:367.
[90] Cited by Robertson.
[91] Dubosq and Lebailly: Compt. Rend. Acad. Sci., 1912, *154*:535.

CHAPTER 34

MEDICAL PARASITOLOGY

By Richard J. Porter, Ph.D.*

Medical parasitology concerns those organisms parasitic in or on man which belong to the animal kingdom. The most important species belong to four phyla of animals, the *Protozoa*, or single-celled animals, the *Platyhelminthes*, or flatworms, the *Nemathelminthes*, or round worms, and the *Arthropoda*. The arthropoda, which include crustacea, insects, mites, spiders, scorpions, etc., are of medical importance primarily as agents of disease transmission and are therefore considered here only insofar as they affect the epidemiology of infections caused by other agents.

Animal parasite infections act fundamentally like infections with other agents. For several reasons, however, the general methods of study differ from those utilized for other infectious organisms. The larger size of animal parasites and their greater visible complexity of structure make morphology a particularly valuable tool in identification. This is especially important because few can be readily cultivated, and physiological bases for their recognition are therefore not generally available. The life-cycles of these organisms are in some cases exceedingly complex, and the elaboration of the complete reproductive histories of many animal parasites has dominated their study. These considerations have obscured the fundamental similarities between infections with animal and plant parasites, and it should be emphasized that differences between some diseases caused by animal parasites, such as amebic dysentery and schistosomiasis, are far greater than their differences from bacterial infections. Because of this great variety among animal parasites, the discussion in the following pages is centered, wherever possible, about representative organisms. It should be borne in mind that many important species will thereby receive less space than they deserve. For fuller discussions of these parasites the reader is referred to the standard texts of parasitology.[1]

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[1] Hegner, Root, Augustine and Huff: *Parasitology*. D. Appleton-Century Co. New York. 1938; Craig and Faust: *Clinical Parasitology*. Lea & Febiger. Philadelphia. 1943; Belding: *Clinical Parasitology*. D. Appleton-Century Co. New York. 1942; Huff: *Manual of Medical Parasitology*. University of Chicago Press. Chicago. 1943; Chandler: *Introduction to Parasitology*. John Wiley & Sons. New York. 1940; Taliaferro: *Immunology of Parasitic Infections*. D. Appleton-Century Co. New York. 1929; Culbertson: *Immunity Against Animal Parasites*. Columbia University Press. New York. 1941. Clinical texts containing much valuable material are Strong: *Stitt's Diagnosis, Prevention and Treatment of Tropical Diseases*. The Blakiston Co. Philadelphia. 1944; and Mackie, *et al.*: *Manual of Tropical Medicine*. W. B. Saunders Co. Philadelphia. 1945.

THE PROTOZOA

The *Protozoa* are animals which carry on all the functions of life in a single cell. They are generally considered analogous to the individual cells composing the higher animals or *Metazoa*, though they are often capable of independent existence under a wider variety of conditions than are metazoan cells. Many thousand species of protozoa have been described, though less than thirty well-defined species are known to parasitize man. They constitute an exceedingly heterogeneous group of organisms, varying in size from that of the larger bacteria to several centimeters in diameter, in complexity of structure from a simple, formless cell like *Endamoeba histolytica* to forms of far greater intricacy than many metazoa, and in life-cycle from the simple binary fission of *Trichomonas vaginalis* to the alternation of hosts and of asexual and sexual reproduction exhibited by the malarial parasites. The following classification, adapted from Wenyon, suggests the diversity of this group:

Sub-phylum Plasmodroma. Movement by thin whip-like filaments called flagella, or by temporary protoplasmic extrusions, pseudopodia.

Class Rhizopoda. Movement entirely or predominantly by pseudopodia. Ex.: *Endamoeba histolytica* of human dysentery.

Class Mastigophora. Movement at least predominantly by flagella. Ex.: *Trypanosoma gambiense* of African sleeping sickness.

Class Cnidosporidia. These produce characteristic spores provided with extrusible filaments. All are parasitic in invertebrates. Ex.: *Nosema bombycis*, agent of Pébrine disease of silkworms, celebrated by the researches of Pasteur.

Class Sporozoa. Reproduction typically by multiple fission (schizogony) alternating with sexual reproduction (sporogony) which gives rise to sporozoites, the infective stage. Alternation of hosts is common. All are parasitic. Ex.: *Plasmodium* species of human malaria.

Sub-phylum Ciliophora. Movement by numerous short fine protoplasmic processes, cilia. Most have two types of nuclei. Ex.: *Balantidium coli* of human dysentery.

Two groups of organisms parasitic in man have not been satisfactorily classified—*Toxoplasma* and *Sarcocystis*.

For material supplementing that presented in the following pages, the reader is referred to the textbooks of protozoology.[2]

THE INTESTINAL AMEBAE (RHIZOPODA)

Endamoeba Histolytica.[3]—Lewis[4] in 1870 and Cunningham[5] in 1871 first reported amebae, probably the non-pathogenic *Endamoeba coli*, in human feces. Lösch[6] in 1875 described what were probably *E. histolytica* in the stools and intestinal ulcers of a fatal case of dysentery. He found

[2] Wenyon: *Protozoology*. Williams & Wilkins Company. Baltimore. 1926; Hegner and Taliaferro: *Human Protozoology*. The Macmillan Co. New York. 1924; Thomson and Robertson: *Protozoology*. Williams & Wilkins Company. Baltimore. 1929; Craig: *Laboratory Diagnosis of Protozoan Diseases*. Lea & Febiger. Philadelphia. 1942.

[3] A valuable comprehensive book is Craig: *Etiology, Diagnosis and Treatment of Amebiasis*. Williams & Wilkins Co. Baltimore. 1944.

[4] Lewis: Appendix to Ann. Rept. of Sanitary Commissioner with Govt. of India, Calcutta, 1870.

[5] Cunningham: Ann. Rept. of Sanitary Commissioner with Govt. of India. Caloutta, 1871, pp. 141–243.

[6] Lösch: Arch. path. Anat., 1875, *65*:196.

similar ulcers containing the amebae in an artificially infected dog. They were first observed in liver abscesses by Koch[7] in 1883, and Kruse and Pasquale[8] in 1894 demonstrated their etiological rôle by producing typical dysentery in cats inoculated rectally with amebae in the bacteriologically sterile pus from a liver abscess.

Characteristics and Life-Cycle.—The active ameba as seen in the intestinal ulcers of dysentery cases is a granular, colorless or pale greenish mass of cytoplasm, 15 to 50 (usually 20 to 30) μ in diameter. It has no definite shape. Locomotion is accomplished by the sudden extrusion of clear projections of cytoplasm, the *pseudopodia,* the remainder of the cell body following these pseudopodia in a flowing motion. The granular cytoplasm often contains red blood cells or debris of tissue cells in various stages of digestion. These are the food engulfed by the ameba. The nucleus may be visible as a delicate ring of granules. Reproduction in this stage is by binary fission, the nucleus undergoing a type of mitosis and the cytoplasm then dividing to produce two daughter amebae like the original. In organisms fixed and stained with hematoxylin the nuclear structure is characteristic, consisting of a thin peripheral layer of fine black granules and a central small black dot, the *karyosome* (Fig. 191): The entire nucleus is generally 4 to 5 μ in diameter. In the lumen of the intestine of a carrier the ameba loses its ingested food particles, shrinks to a diameter of 6 to 20 μ, rounds up and becomes essentially non-motile, sending out only an occasional pseudopodium. This is the *precystic stage,* which soon secretes about itself a clear wall, becoming the partially resistant *cyst.* In its passage down the intestine the cyst continues to develop, acquiring a vacuole of glycogen and one or more ovoid rods of black-staining material, the *chromatoid bodies.* The nucleus divides into two and then four, all resembling the nucleus of the active ameboid stage though considerably smaller. In the mature cyst the glycogen vacuole soon disappears, and the chromatoid bodies persist at most for a few days.

The cyst is the infective stage, since it alone can pass through the stomach without destruction. As studied in culture its excystment consists of the emergence of a four-nucleate organism which by a complicated division process produces eight small amebae. These presumably are the stages which enter the tissues of the intestinal wall to initiate a new infection.

Barret and Smith[9] cultivated a parasitic ameba of turtles in a mixture of serum and physiological saline solution. The first culture of *E. histolytica* was performed by Boeck and Drbohlav[10] in equal parts of inactivated human serum and Locke's solution tubed over egg slants. Later modifications have substituted other types of serum, Ringer's solution or physiological saline, and other slant media. Slants may be omitted for short-term cultivation.[11] Pure cultures of *E. histolytica* have not been obtained,

[7] Koch and Gaffky: Arb. a. d. kaiserl. Gesundh., 1887, *3*:1.

[8] Kruse and Pasquale: Ztschr. f. Hyg., 1894, *16*:1.

[9] Barret and Smith: Amer. Jour. Hyg., 1923, *3*:205; 1924, *4*:155.

[10] Boeck and Drbohlav: Proc. Nat. Acad. Sci., 1925, *2*:235; Amer. Jour. Hyg., 1925, *5*:371.

[11] Craig: *Laboratory Diagnosis of Protozoan Diseases.*

the amebae growing only in the presence of bacteria. Unlike the amebae in their normal habitat, they ingest bacteria, yeasts and starch grains. Cysts are formed in certain media containing added rice starch. Some re-

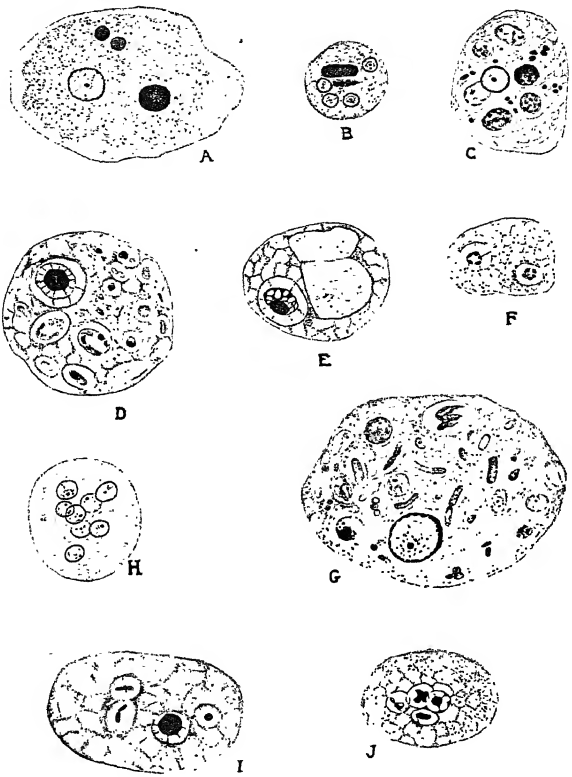


Fig. 191.—The amebae living in man. *A*. Active ameboid form of *Endamoeba histolytica* containing three red blood cells. *B*, Mature quadrinucleate cyst of the same containing two chromatoids. *C*, Active ameboid form of *Endamoeba gingivalis*. *D*, Active ameboid form of *Iodamoeba williamsi* containing many intestinal bacteria. *E*, Cyst of same showing large double vacuole which in life was filled with glycogen. *F*, Ameboid form of *Dientamoeba fragilis* containing two nuclei. *G*, Large active ameboid form of *Endamoeba coli* containing intestinal bacteria and débris. *H*, Mature octonucleate cyst of same. *I*, Ameboid form of *Endolimax nana*. *J*, Mature quadrinucleate cyst of same. *A*, *B*, *C*, *G*, and *H* × about 1300 (Dobell). *D*, *E*, *F*, and *J* × 3000 (Taliaferro and Becker). *I* × 3000 (Taliaferro in Hegner and Taliaferro's *Human Protozoology*, courtesy of The Macmillan Company).

semble those formed naturally in the intestine, but others are abnormal, containing as many as thirty nuclei.

Amebiasis and Amebic Dysentery.—The amebae normally inhabit the mucosa and submucosa of the large intestine. By means of enzymes

and mechanical action they digest the tissues to produce minute necrotic lesions of the mucosa, which may enlarge into more or less extensive undermined ulcers in the submucosa. The abundance and severity of the lesions determine the clinical picture. Clinical cases range from moderate diarrhea to acute dysentery with passage of blood and mucus, extreme weakness and, not infrequently, death. A varying proportion, probably between 20 and 50 per cent, of individuals with amebic dysentery shows necrotic abscesses of the liver. These also occur occasionally in individuals who have never had intestinal symptoms of infection. Lung abscesses, often produced by extension from the liver through the diaphragm, occur in a small number of individuals, and abscesses have been reported rarely in practically every organ of the body.

The great majority of infected individuals are carriers exhibiting no symptoms. While the possibility exists, though the evidence is still inconclusive, that there are strains of *E. histolytica* differing in virulence, it was shown by Walker and Sellards[12] that a strain which produces acute dysentery in some individuals may give rise to the symptomless carrier condition in others. This has generally been attributed to differences in natural resistance of infected individuals, but recent evidence suggests that associated bacteria significantly influence the severity of the amebic lesions.[13] While the factors in virulence of the infection are unknown, it is clear that the relationship between *E. histolytica* and its host is exceedingly complex.

IMMUNITY.—There is no direct evidence of acquired immunity to *Endamoeba histolytica* infection. However, the marked reduction in incidence above middle life suggests that some resistance develops. Natives of hyperendemic regions show acute dysentery less frequently than aliens, possibly as a result of acquired immunity. Serum antibody response to the infection is evidenced by the complement fixation test, which is positive in over 90 per cent of infected individuals, becoming negative after successful elimination of the amebae by treatment.

DIAGNOSIS.—Diagnosis of infection with *E. histolytica* depends upon the finding of characteristic organism in the stools and their morphological differentiation from the non-pathogenic amebae occurring in human feces (p. 706). Cultivation has been utilized, but in cultures *E. histolytica* resembles the non-pathogenic *E. coli* so closely that differential diagnosis is often very difficult. Direct smears of fresh loose stools in warm saline may reveal the active ameboid stages. They may also be found in material taken directly from lesions in the lower bowel with the aid of the proctoscope. In well-formed stools cysts may be expected, and these are most easily identified in iodine solutions, such as Lugol's iodine. When cysts are present they may be concentrated by the method of zinc sulfate flotation, in which they rise to the surface of a solution of high specific gravity. This method reveals many infections which are missed by direct examination. *E. histolytica* appears irregularly in the stools and repeated examinations are often necessary to establish a diagnosis. It should be re-emphasized that recovery of the organisms is only the first step in diagnosis of

[12] Walker and Sellards: Philippine Jour. Sci. (B), 1913, *8*:253.
[13] *Cf.* Deschiens: Ann. Inst. Pasteur, 1938, *61*:5.

E. histolytica infection. The final step is the identification of the parasites, a task requiring abundant experience.

Emetine, a vegetable alkaloid, was long used in treatment of amebiasis. While it usually has a favorable effect on the symptoms and is of value in amebic liver abscess, it rarely eliminates the infection. Several iodine-containing drugs, notably chiniofon and diodoquin, and arsenicals, especially carbarsone, are highly effective in eradicating the amebae.

Epidemiology and Control.—The active ameboid stages of *E. histolytica* die quickly after exit from the body, being highly susceptible to drying and to changes in temperature and salt concentration. Since they are rapidly destroyed by gastric juice they are not infective when swallowed. The amebic dysentery patient is therefore practically harmless as a source of infection, since only the ameboid stages occur in his stools. The cysts passed by carriers, while not at all comparable to bacterial spores in resistance, show considerably less susceptibility to conditions outside the body than do the ameboid stages. Early reports indicated survival of as long as several months. These data were obtained with a test for viability based on the belief that cysts which did not stain with eosin were alive. The studies since 1925, ultilizing cultivation as a test for viability, have given much shorter survival times, seventeen days in water at 0° C., nine to ten days in feces or water at room temperature, and thirty minutes at 45° C. The cysts are quickly killed at 50° C. Early reports indicated that cysts of *E. histolytica* were unharmed by concentrations of free chlorine over one hundred times as great as those used in water purification. Under the conditions of these tests, in which the organic content of the water is far higher than that encountered in water purification plants, recent reports indicate that the cysts of *E. histolytica* are no more resistant to chlorine than are such intestinal bacteria as *Bact. coli.*[14] It is not known at present whether this relationship holds under normal water treatment conditions. In this connection it should be borne in mind that in the Chicago amebic dysentery outbreak of 1933 there was abundant circumstantial evidence that the infection was spread by water containing sufficient residual chlorine to kill intestinal bacteria.[15]

In general, the spread of *E. histolytica* resembles that of intestinal bacterial infections, utilizing any means by which fecal contamination reaches the human mouth. Most important are drinking water, food-handlers and houseflies. Swimming pools, though not definitely incriminated, are a potential source of infection. Viable cysts have been found in the droppings of houseflies one to two days after exposure, and flies have been claimed to be responsible for at least one important outbreak. The 1933 epidemic in Chicago referred to above was traced to local sewage contamination of drinking water in two hotels. This epidemic, in which 1409 cases were discovered and there were 98 deaths, was the first important outbreak in a city of the temperate zone. It directed medical attention to a problem which had been considered important only in the tropics.

The distribution of *E. histolytica* is world-wide, but temperate regions

[14] *Cf.* Kessel *et al.:* Amer. Jour. Trop. Med., 1944, *24:*177.

[15] McCoy *et al.: Epidemic Amebic Dysentery*. Nat. Inst. Health Bull. No. 166. 1936.

have generally a low incidence of infection. Surveys indicate a general infection rate in the United States of about 10 per cent, though in some southern localities the incidence has approached 40 per cent. In the tropics the carrier rate is generally very high, often exceeding 50 per cent. Human carriers are the only important source of infection. Although various animals, especially dogs and cats, are susceptible to infection, and amebae morphologically identical with *E. histolytica* occur naturally in lower animals, particularly rats, dogs and monkeys, there is no epidemiological evidence that human infections have been derived from these sources. Furthermore, the evidence that these amebae are *E. histolytica* is not conclusive. It is well-known that strains of *E. histolytica* differ greatly in cyst size, and there have long been claims that the strains with small cysts (6 to 10 μ in diameter) are rarely or never pathogenic.[16] These possibilities, though not conclusively demonstrated, are of the utmost importance in the epidemiology of amebiasis.

Control of the spread of *E. histolytica* is not significantly different from that of other human enteric infections. The high incidence of carriers not known to have had clinical dysentery complicates the problem, but it is ultimately a matter of prevention of access of human fęces to the mouths of susceptibles.

Other Species of Amebae Parasitic in Man.—Four other species of amebae live in the human intestine (Fig. 191). Being non-pathogenic they are of medical interest only because they must be ruled out in the diagnosis of *E. histolytica*. *Endamoeba coli*, a common species, occurring in 25 to 50 per cent of the general population of the United States, differs from *E. histolytica* in several characters. The stained nucleus shows thicker peripheral chromatin blocks and a larger and usually non-central karyosome. The ameboid stage is sluggishly and usually non-progressively motile with blunt, slowly extruded pseudopodia. It ingests bacteria and other particles but rarely red blood cells. The spherical cysts average somewhat larger, 10 to 33 μ in diameter, contain eight nuclei in the mature stage, and may show chromatoid bodies with pointed or "splintered" ends. *Endolimax nana*, present in about 25 per cent of the United States population, is smaller, 6 to 15 μ in diameter in the ameboid stage. The stained nucleus shows no peripheral chromatin but a very large, nearly central karyosome. Movement is sluggishly progressive and bacteria are ingested. The spherical or ovoid cyst is 5 to 14 μ in diameter, containing one to four minute nuclei and sometimes small spherical or rod-like chromatoid bodies. *Iodamoeba williamsi*, present in 10 to 15 per cent of the United States population, measures 8 to 20 μ in diameter in the ameboid stage. The stained nucleus shows a large central karyosome surrounded by a layer of granules. Movement and inclusions are like those of *E. coli*. The cyst is irregular in shape, 5 to 20 μ in diameter, and contains one or rarely two nuclei. Minute granules may be seen, but the most striking feature of the cyst is a large glycogen mass, staining dark brown with iodine.

Dientamoeba fragilis, a rare parasite in the general population though sometimes common in institutions, is a very small form, 5 to 12 μ in diam-

[16] *Cf.* Brumpt: *Précis de Parasitologie.* Masson & Cie. Paris. 1936.

eter. It usually shows two nuclei, each containing a large multiple karyosome. It moves actively, ingesting bacteria. No cyst stage is known, the ameboid stage apparently being responsible for transfer. Dobell[17] has recently suggested, principally on the basis of its nuclear structure, that *D. fragilis* is a degenerate type of flagellate rather than a true ameba.

Endamoeba gingivalis, probably the first parasitic ameba seen, was reported by Gros[18] in 1849 from the tartar between the teeth. It has no known cyst stage, apparently being transmitted in the ameboid stage by contact. Formerly suspected of an etiological role in pyorrhea, it is now considered harmless.

CILIOPHORA

Balantidium coli is a large parasite of the intestine of man, monkeys and the pig, usually 50 to 80 μ long by 40 to 60 μ in breadth (Fig. 192). Human infection is widespread but rare. Most of the known human cases

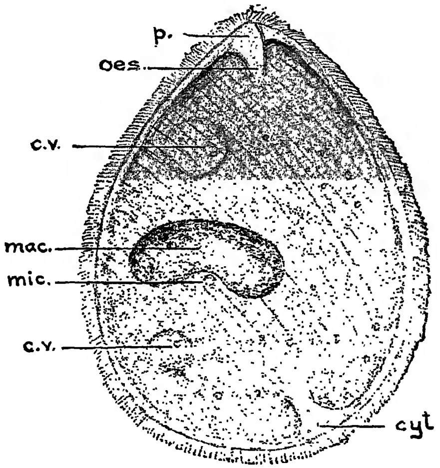


Fig. 192.—*Balantidium coli* from man. c.v., Contractile vacuole; cyt., cytopyge; mac., macronucleus; mic., micronucleus; oes., esophagus; p., peristome (Hegner in Hegner and Taliaferro's *Human Protozoology*, courtesy of The Macmillan Company).

show diarrhea or dysentery pathologically like that produced by *E. histolytica*, but carrier infections have been reported.[19] The pig is probably the usual source of human infection, passing resistant cysts which are infective when ingested with contaminated food or water. A variety of laboratory animals, including cats and monkeys, are susceptible, and cultivation has been accomplished in media similar to those used for *E. histolytica*. Carbarsone is said to eliminate infection.[19] Although several other species of ciliates have been reported from human stools, it is not certain that they were human parasites.

[17] Dobell: Parasitology, 1940, *32*:417.
[18] Gros: Bull. Soc. Imp. Nat., Moscow, 1849, 22 (1 part): 549.
[19] Young: Jour. Amer. Med. Assn., 1939, *113*:580.

INTESTINAL FLAGELLATES (MASTIGOPHORA)

A variety of species of *Mastigophora* occurs in the human intestine (Fig. 193). The common species are *Chilomastix mesnili*, *Trichomonas hominis* and *Giardia lamblia*. Only the last is of medical importance. It is generally believed to produce a diarrhea in children and occasionally in adults. The evidence is dubious, the best indication being the fact that atabrine, which eliminates the organism, often cures the intestinal condition.

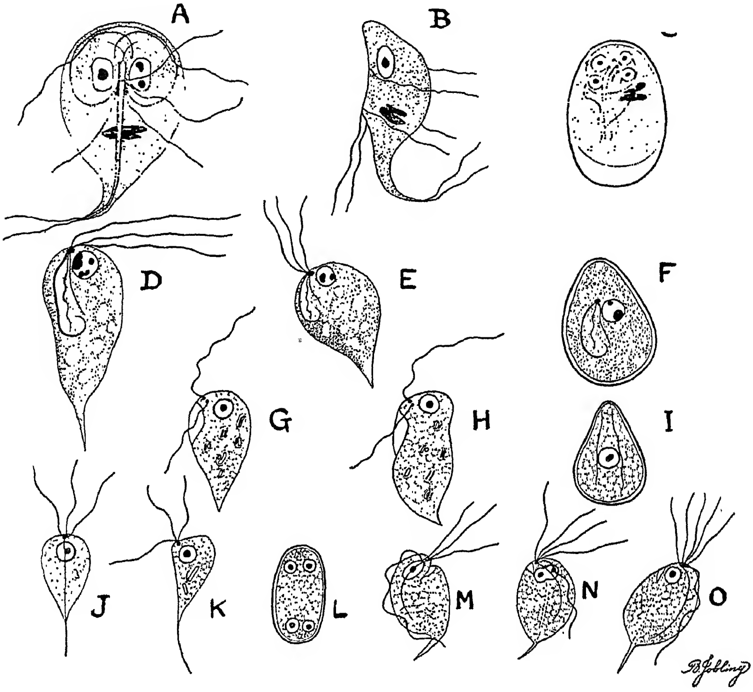


Fig. 193.—The flagellates of the human intestine; × 2000. A–C, *Giardia lamblia*, free and encysted forms. D–F, *Chilomastix mesnili*, free and encysted forms. G–I, *Embadomonas intestinalis*, free and encysted forms. J–L, *Tricercomonas intestinalis*, free and encysted forms. M–O, *Trichomonas hominis*, forms with three, four, and five flagella (Wenyon, Proceedings of Royal Institution of Great Britain, March 3, 1922).

Trichomonas elongata, often referred to as *T. buccalis*, is a common non-pathogen of the mouth. *T. vaginalis*, occurring in the human vagina, and rarely in the male urethra, is often associated with vaginitis. The evidence for its pathogenesis is suggestive but inconclusive.

Chilomastix and *Giardia* are spread by cysts from human feces. No cysts are known for *Trichomonas*, and the relatively resistant flagellated stages are apparently responsible for dissemination. *Trichomonas* species and *Chilomastix* grow on various media, but *Giardia* has not been successfully cultivated.

HEMOFLAGELLATES (MASTIGOPHORA)

The family trypanosomidae, commonly called hemoflagellates because it includes the mastigophora of the blood and other tissues, comprises several genera which are parasites of vertebrates, invertebrates or plants. They are classified on the basis of the morphological types each exhibits. Figure 194 illustrates these morphological types, of which *C* may be taken as an example, since it is the simplest. This form is an elongated blade-like cell body, the cytoplasm staining blue with blood stains such as Giemsa. Near the center is a round reddish-violet-staining body, the nucleus. Arising from a small, dark purple-staining granule, the blepharoplast, at the anterior end is a long whip-like organ of locomotion, the flagellum. Near the blepharoplast is a larger body, staining similarly, the parabasal body. This stage of the parasite is known as the *simple flagellate.** In some forms the simple flagellate stage may round up and lose its flagellum to become

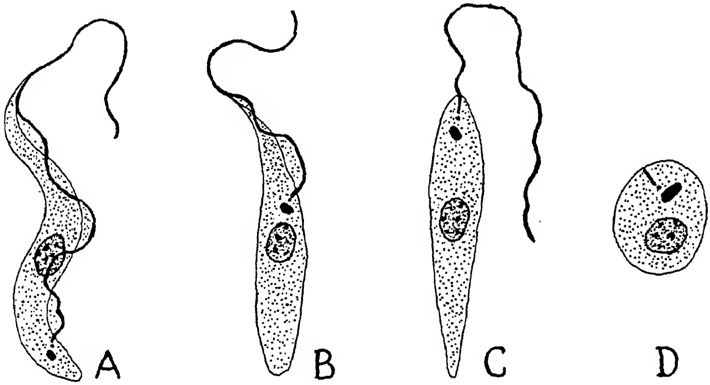


Fig. 194.—Morphological types of hemoflagellates. *A*, trypaniform stage. *B*, Intermediate flagellate. *C*, Simple flagellate. *D*, Non-flagellate stage.

the *nonflagellate* stage shown at *D*. On the other hand it may metamorphose into the *intermediate* stage (*B*) by posterior migration of the blepharoplast to near the nucleus. In this stage the flagellar filament is connected to the body proper by a thin film of cytoplasm, the undulating membrane, which waves with the flagellar movement, thus acting as a fin. The *trypaniform* stage (*A*) is attained by further migration of the blepharoplast to near the posterior end with production of an undulating membrane extending the whole length of the cell.

The medically important groups of hemoflagellates fall into two genera. *Trypanosoma gambiense* and related forms exhibit the trypaniform stage in the vertebrate host and trypaniform and intermediate stages in the invertebrate. *Trypanosoma cruzi* exhibits all four stages. Members of the

* Simplified terms are here substituted for the confusing names in general use. Most texts designate type A *trypanosome*, type B *crithidia*, type C *leptomonas* or *herpetomonas*, and type D *leishmania*.

genus *Leishmania* exhibit the non-flagellate stage in the vertebrate and the simple flagellate in the invertebrate host. Reproduction of all forms is by binary fission. The nucleus, blepharoplast and parabasal body divide, a new flagellum arises from one blepharoplast, and the cytoplasm divides longitudinally to produce two daughter cells. In the species with which we are concerned, the non-flagellate stages are intracellular in the vertebrate host. The flagellated stages inhabit body fluids of vertebrates or the alimentary tract of insects.

The Trypanosomes

Trypanosoma Gambiense.—Forde[20] observed, in the blood of a Gambian native, a flagellate which was described by Dutton[21] in 1902 as *T. gambiense*. In 1903, Castellani[22] observed similar flagellates in the cerebrospinal fluid of a sleeping sickness patient in Uganda. Bruce and Nabarro,[23] in 1903, transmitted *T. gambiense* to monkeys with a tsetse fly, and Kleine,[24] in 1909, showed that the parasites underwent a cyclic development in the fly.

Fig. 195.—*Trypanosoma gambiense* in stained blood film; × about 2000. (Kraal.)

Characteristics and Life-Cycle.—In the blood and lymph of early cases of African sleeping sickness *T. gambiense* exhibits the trypaniform stage, which in fresh preparations may be seen wriggling among the erythrocytes (Fig. 195). It measures 15 to 40 μ in length, varying from short, broad forms with no free flagellum beyond the undulating membrane to long thin forms with a free flagellum. Reproduction, as in all members of the family, is by binary fission. The transmitting insect, a tsetse fly, becomes infected by ingestion of the parasites in infected blood. In the stomach of the fly those flagellates which survive multiply as trypaniform and intermediate stages, first in the crop, stomach and intestine and later in the salivary glands. Here they form infective parasites, which are injected by the bite of the fly twenty days or more after its infecting blood meal.

[20] Forde: Jour. Trop. Med. & Hyg., 1902, *5*:261.
[21] Dutton: Thompson-Yate's Lab. Reports, 1902, *4*:455.
[22] Castellani: Brit. Med. Jour., 1903, p. 1218.
[23] Bruce and Nabarro: Rep. Sleeping Sickness Comm. Roy. Soc., 1903, *1*:11.
[24] Kleine: Deut. med. Wchnschr., 1909, *35*:469, 924.

On NNN medium,[25] a concentrated blood agar, the parasites multiply to a limited extent. Continuous cultivation has been achieved in some instances, but it is too difficult to be of practical value. Abundant multiplication occurs on the chorio-allantoic membrane of the developing chick embryo.

African Sleeping Sickness.—The incubation period following an infective tsetse fly bite varies from two or three weeks to several months. The first stage of the human disease is a systemic infection in which the parasites are found chiefly in the blood but also in the lymph. Irregular fever is generally the first symptom. Somewhat later, as the parasites come to predominate in the lymph nodes, these organs and the spleen are enlarged, and anemia and wasting are seen. Cardiac injury may be prominent, and edema is often present. This stage gradually gives way to the sleeping sickness stage, in which the parasites are most abundant in the cerebrospinal fluid, occurring less commonly in the lymph. The central nervous system exhibits the prominent lesions, typically resulting in somnolence, apathy and weakness, less commonly in mania and other violent manifestations. Coma and death are the final outcome. Typically the course is of several months' duration, but in parts of East Africa the disease develops more rapidly, invasion of the central nervous system occurs early, and death frequently results from cardiac injury before the typical sleeping sickness stage is reached. In the blood of laboratory animals infected from cases in this area about 5 per cent of the flagellates are short, showing posterior nuclei; this is true only rarely in the parasites from other areas. Because of these differences in morphology and pathogenicity and the fact that transmission involves different species of tsetse flies, Stephens and Fantham[26] separated these parasites as a different species, *T. rhodesiense*. Most authors now consider this a local race of *T. gambiense*.

Immunity.—Spontaneous recovery is said to occur occasionally in African sleeping sickness. Except for this there are no data available on natural or acquired immunity in man. In rats and mice *T. gambiense* and related parasites produce a rapidly fatal parasitemia, while in guinea pigs and some other hosts they show a characteristic relapsing type of infection. After a period of unchecked multiplication, the organisms are rapidly destroyed, only to reappear again in a few days and repeat the cycle. An antibody arising in the serum of a guinea pig at the time of crisis destroys parasites of the strain present before the crisis, while the strain present after flagellates have reappeared again is resistant to this antibody. This phenomenon has been observed in animals infected originally with a single parasite, indicating that the change in antigenic structure cannot depend on selection alone but must involve adaptive modification of the parasite.

Diagnosis.—Various serum reactions occur, but are not dependable for diagnosis. Laboratory diagnosis depends on the finding and morphological identification of the organisms in body fluids, blood, lymph node juice, or cerebrospinal fluid. Dry smears stained with blood stains, such as Wright's, are used, either of the whole fluid as obtained or of centrifuged specimens.

[25] *Cf.* Craig: *Laboratory Diagnosis of Protozoan Diseases*, Phila., 1942.
[26] Stephens and Fantham: Proc. Roy. Soc. London, 1910 (B), *83:*28, *85:*223.

In the case of blood, thick or thin films (see p. 725) may be used. The parasites are usually scarce, and a negative finding in any one of the body fluids is of little significance.

Epidemiology and Control.—Rare instances of probable coital infection have been reported. However, the normal transfer of infection occurs by the bite of tsetse flies as described above (p. 711). Two species of flies are of most importance, *Glossina palpalis* in West Africa and *G. morsitans* in East Africa, where the more virulent Rhodesian form of the disease occurs. Other species of importance are *G. tachinoides*, *G. swynnertoni* and *G. pallidipes*. These insects are close relatives of the common house fly. They are limited to tropical Africa and a small area in South Arabia, and the human disease is confined to their range. They resemble the house fly in appearance except for the long, narrow proboscis, which is held straight forward from the head, and the manner of folding the wings at rest, flat on the back with one directly above the other. They range in size from that of a house fly to two or three times this size. Both males and females bite and can transmit the disease. They bite exclusively by day. The larvae develop completely in the body of the female, are deposited singly on loose soil or sand in well-shaded places, and quickly burrow into the soil to pupate. After four to eight weeks the adults emerge. *G. palpalis* breeds almost entirely near water and is thus more limited in local distribution than *G. morsitans*, which is less dependent on shade and moisture.

The significance of wild animals in spread of African sleeping sickness has long been disputed. Various large wild animals, especially the situtunga antelope, harbor the flagellates, usually without symptoms, and they have been shown to maintain the infection for long periods in the absence of human reservoirs. However, most authorities agree that man is usually the source of infection. The incidence of human infection varies widely. It is not commonly higher than 2 per cent at present, though in the past villages were observed with infection rates as high as 50 per cent, and catastrophic epidemics have occurred.

The variety of control methods in use testifies to their relative ineffectiveness. Several valuable drugs are available for treatment of the human infection, a method whose success is proportional to the local significance of man as a reservoir of infection. Tryparsamide and other arsenicals are effective in all stages of the disease, though less so in the Rhodesian form, wher Bayer 205 (Germanin, Fourneau 309) is more active. This drug is useless in sleeping sickness, but is effective during the first stage and has the particularly valuable property of protecting against infection for at least three months after administration. A new series of compounds represented by stilbamidine and pentamidine shows genuine promise. Extensive control programs are under way with mass treatment of diagnosed human cases and their value seems well demonstrated. In some areas mass destruction of the game reservoir has been attempted, but the success of this measure is doubtful. Where the human and animal infection rates are particularly high, wholesale removal of human populations has been carried out. Control of migrants, a perpetual problem in much of Africa, is utilized in some districts.

The life cycle and complex behavior of the flies make their control exceedingly difficult. Traps and hand-catching have greatly reduced the fly population in some regions. Inspection and fumigation of vehicles have been used to limit the spread of tsetse. The best single method is clearing of forest and brush, particularly along streams and around villages, which destroys the breeding and resting places of the flies.

Trypanosoma Cruzi.—Chagas[27] discovered intermediate stage flagellates in the hind gut of the bug *Triatoma megista* in Brazil. He showed their infectivity for mammals and later found trypaniform stages in children with a characteristic disease, now known as Chagas' disease. He described the organism as *Schizotrypanum cruzi*, having misinterpreted the reproduction in man and animals as schizogony. It is now called *Trypanosoma cruzi* by most authors.

Characteristics and Life-Cycle.—The parasites as seen in the blood of early cases of Chagas' disease are of the trypaniform type, fundamentally similar to those of *T. gambiense* but smaller, about 20 μ in length, and with

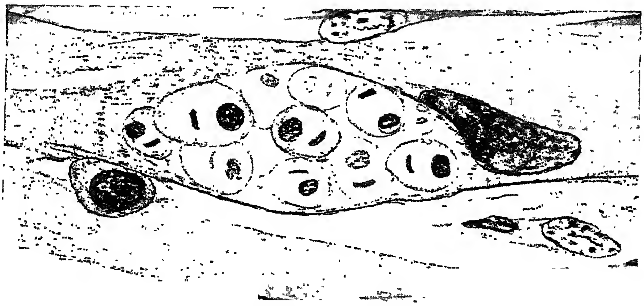


Fig. 196.—Non-flagellate form of *Trypanosoma cruzi* in human heart muscle; × 3700 (Taliaferro in Hegner and Taliaferro's *Human Protozoology*, courtesy of The Macmillan Company).

the posterior end pointed. No multiplication occurs in the blood. The predominant phase in man and experimental animals is a small, rounded non-flagellate stage, 3 to 5 μ in diameter, occurring in clumps in various tissue cells—heart muscle, macrophages and endothelium (Fig. 196). These organisms have a nucleus and parabasal body as described above (p. 709). They multiply in the infected cells by binary fission, producing the dense clumps of organisms mistaken by Chagas for stages of schizogony. Small numbers of trypaniform stages are apparently continually produced and shed into the circulating blood from the nests of intracellular non-flagellate parasites.

Various bugs of the family Reduviides acquire the infection from a blood meal containing the trypaniform stages. These become intermediate flagellates in the mid- and hind-gut, where they multiply to produce infective trypaniform flagellates two to three weeks after the infecting blood meal. These organisms are shed in the feces of the bug. They infect man by enter-

[27] Chagas: Mem. Inst. Oswaldo Cruz, 1909, *1*:159.

ing the wound caused by the bite of the infected bug or by penetrating the mucosae, particularly of the mouth or eye.

Continuous culture of *T. cruzi* on NNN medium and in simpler broth media is relatively easy. The parasite multiplies in the water of condensation as trypaniform and, predominantly, intermediate flagellates.

Chagas' Disease.—Chagas described the infection from a region having serious endemic goiter, and most of his cases had marked thyroid pathology. It has since been shown in other regions that the goitrous manifestations he described are not part of Chagas' disease. The incubation period is one to two weeks. Irregular fever and edema, particularly of the eyelids (Romaña's sign) characterize the acute phase, and there is considerable enlargement of lymph nodes, spleen and liver toward the end of this period. The acute disease is rare except in small children, in whom occur almost the only deaths attributable to the infection. The chronic disease, which occurs in adults or following the acute stage in children, varies in symptomatology with the localization of the parasites but is most often characterized by myocarditis caused by heart muscle infection.

Immunity.—Evidence concerning acquired immunity is lacking in the human disease, but experimental animals are immune to reinfection after recovery. Serum from such animals partially protects against experimental infection. The serum of human cases fixes complement in the presence of extracts of infected tissues or cultures of *T. cruzi*, and the complement fixation test, the Machado reaction and various modifications, are widely used for laboratory diagnosis.

Diagnosis.—Other laboratory diagnostic methods depend on the demonstration of the parasites. During the early stages of the human disease trypaniform flagellates may be found in stained blood smears. Later, indirect evidence of the presence of parasites may be obtained by blood culture or by "xenodiagnosis" ("host diagnosis"), in which laboratory-reared bugs become infected after feeding on a case.

Epidemiology and Control.—Natural infection is acquired from infected bugs as described above. The chief vectors are *Triatoma* (*Mestor*) *megista* and *Rhodnius prolixus*, but about forty species of the family Reduviidae have been incriminated. These insects are members of the order Hemiptera, or True Bugs, to which belong also bed bugs, chinch bugs and many others. They are large insects with an elongated, cone-shaped head. Most species are predatory on other insects but some live on vertebrate blood. They inhabit the nests of various animals and may occur in human houses of poor construction, where males and females commonly bite sleeping persons about the mouth or eyes. The total life-cycle, involving egg, larval, nymphal and adult stages, occupies six to ten months.

The human disease is widespread in South and Central America but of low incidence in most areas. Various mammals are susceptible and the infection occurs naturally in many of these, particularly dogs, cats, wild rodents, opossums and armadillos, all of which serve as reservoirs of human infection. In the southwestern United States natural infections occur in the insects and in the wild rodents which they infest, but human infections have not been reported in this country.

Successful control of Chagas' disease has not been attained. The most promising methods involve destruction of infected domestic animals and improvement of human houses to exclude the insects and the reservoir hosts.

There is no successful chemotherapy for Chagas' disease, the drugs useful in African trypanosomiasis being inactive in this infection.

Other Species of Trypanosoma.—A number of important diseases of domestic animals are caused by species of *Trypanosoma* similar to *T. gambiense. Nagana* is a rapidly fatal disease of the horse family, and to a less extent of cattle and dogs, occurring in a wide area of East Africa. It is caused by *T. brucei*, an organism very similar to the Rhodesian form of *T. gambiense*, and transmitted mostly by *G. morsitans*. Wild game are commonly infected without detectable symptoms and are an important reservoir. A number of other species transmitted by tsetse flies are important disease agents of animals in Africa. *T. evansi* causes a disease of horses, camels and mules known as *surra*, which is widespread in Asia, extending to Russia, Arabia and Madagascar. It is transmitted mechanically by horse flies and stable flies. Related species cause equine diseases in South America, one of which, *T. hippicum*, is transmitted by vampire bats. *T. equiperdum* causes a disease of the horse family known as *dourine*, of nearly world-wide distribution. The disease is known as "horse syphilis" because of a general similarity in course of infection and the fact that spread occurs by coitus.

Two rodent species, *T. lewisi* of rats and *T. duttoni* of mice, are of especial interest. They are found in various parts of the world and are easily obtained for laboratory study. Like *T. gambiense* they occur in the vertebrate host only in the trypaniform stage. They resemble *T. cruzi*, however, in that multiplication in the invertebrate hosts, fleas and sucking lice, takes place in the mid- and hind-gut, and infection of the vertebrate is acquired from flagellates in the insects' feces. Host specificity is very strict, and although the parasites are readily cultivable on NNN medium they do not multiply appreciably in developing chick embryos as do flagellates of the *T. gambiense* type. In rats inoculated with blood containing *T. lewisi* the flagellates multiply unchecked for several days, showing the great size variation characteristic of rapidly reproducing populations. This variation decreases rapidly with the appearance of a non-absorbable antibody, an *ablastin*, which completely inhibits reproduction. Within a few days a trypanolysin destroys a majority of the parasites, the remainder persisting in the peripheral blood without multiplication until, after one to several months, another trypanolysin eliminates the survivors.[28]

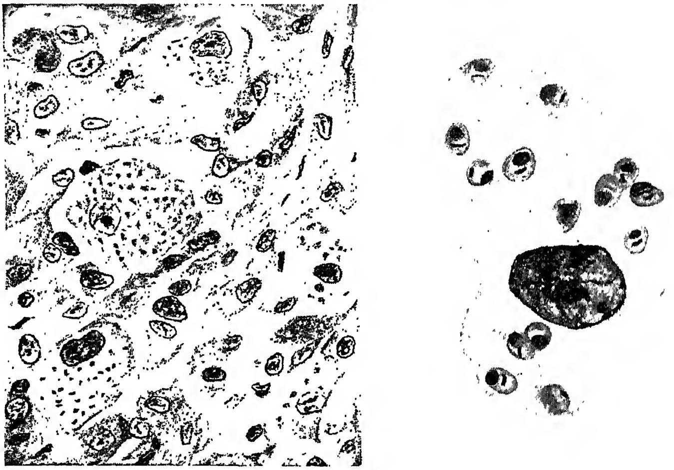
The Leishmanias

Leishmania Donovani.—Leishman[29] and Donovan,[30] in 1903, described oval parasites of the macrophages in cases of kala-azar in India.

[28] Taliaferro: Jour. Exp. Med., 1924, *39*:171.
[29] Leishman: Brit. Med. Jour., 1903, p. 1252.
[30] Donovan: Brit. Med. Jour., 1903, p. 1401.

These were recognized as mastigophora when Rogers[31] showed they developed into motile flagellates in culture.

Characteristics and Life-Cycle.—In the human disease, kala-azar, the parasites occur as non-flagellate stages, 3 to 5 μ long, in macrophages, where they resemble the non-flagellate stages of *T. cruzi* (Fig. 197). They multiply by binary fission until the cytoplasm of the host cell is crowded, when they escape to infect new cells. While the parasites predominate in internal organs, they occur in the skin macrophages as well, and it is probably from this site that the intermediate hosts, sandflies of the genus *Phlebotomus*, become infected. The parasites acquire the simple flagellate form, 14 to 20 μ long, and multiply in the mid- and fore-gut of the insect, which



A *B*

Fig. 197.—*Leishmania donovani* in the human spleen. *A*, Section of spleen showing five macrophages infected with the parasites. *B*, One macrophage enlarged to show structure of the parasites. *A*, × about 550; *B*, × 2500 (Taliaferro in Hegner and Taliaferro's *Human Protozoology*, courtesy of The Macmillan Company).

becomes infective after a week or more. The simple flagellates, injected by the bite of the fly into a new host, reestablish the vertebrate phase of the cycle.

L. donovani is easily cultivated in NNN and other media at 22 to 35° C., multiplying in the simple flagellate stage as in the insect host (Fig. 198).

Kala-Azar.—Visceral leishmaniasis, or kala-azar, is usually a chronic disease. Typically it begins, after an incubation period of one to four months (sometimes much longer), with a high temperature, which merges into an irregular fever showing long swings of temperature. The spleen and liver enlarge greatly with hyperplasia of the parasitized macrophage system. Wasting, emaciation and edema of the legs are common. Dysentery often occurs as a result of heavy infection in the intestinal wall. The skin

[31] Rogers: Lancet, 1904, p. 215.

is typically dusky in hue, whence the name kala-azar, meaning "black fever." The skin is infected with the parasite but usually does not show lesions until months after systemic recovery when depigmented areas appear, later often becoming slightly raised papules. Anemia and leucopenia are characteristic. Death is the rule in untreated cases, usually as a result of secondary infection.

IMMUNITY.—Treated or spontaneously cured human infections are apparently followed by a lasting, solid immunity, for second infections are exceedingly rare. However, vaccines have failed to protect against or ameliorate the disease. Complement-fixing and agglutinating antibodies are detectable in active and recovered cases, but they give cross-reactions with cutaneous leishmaniasis.

DIAGNOSIS AND TREATMENT.—Crucial laboratory diagnosis may be obtained by the finding of parasites in biopsies of skin, spleen, liver or bone

Fig. 198.—*Leishmania donovani*; simple flagellate forms, from a culture; Wright's stain; × 1800 (Bulletin No. 1, Office of the Surgeon General, Washington, January, 1913).

marrow. Sternal bone marrow puncture is a reliable and safe procedure. Blood cultures may be positive. In addition, a group of non-specific serological reactions has shown great value. These reactions depend on the fact that the serum euglobulin is greatly increased in amount. In the formol-gel test (Napier's aldehyde reaction), positive sera form an opaque gel when formalin is added. In the antimony test a pentavalent antimonial drug causes a heavy flocculent precipitate in sera of cases. These tests are positive in over 80 per cent of cases and give false positives only occasionally with other diseases, especially schistosomiasis and malaria.

Several antimony compounds, particularly neostibosan and solustibosan, are effective in kala-azar. Treatment is often followed, after several months, by dermal leishmaniasis, which may persist for several months. The drugs stilbamidine and pentamidine mentioned in connection with African sleeping sickness (p. 712) are also effective in kala-azar.

Epidemiology and Control.—Kala-azar occurs in China, India, South

Russia, Mesopotamia, the Mediterranean littoral, Equatorial Africa, Eastern Brazil and Northern Argentina. In Asia it is apparently a disease of man, though various lower animals, particularly dogs, hamsters and mice, are susceptible. Infection may occur at any age but is most common in older children and young adults. In the Mediterranean area, however, it is primarily a disease of small children. Here dogs are commonly infected and apparently serve as the reservoir, since, unlike children, they show abundant parasites in the skin. The Mediterranean parasite is called by some authors *L. infantum* and the South American form has been described as *L. chagasi*, but they are generally believed to belong to the species *L. donovani*.

Factors of seasonal and geographical distribution early pointed to sandflies as probable vectors, and it was soon shown that they were susceptible, developing heavy intestinal infections when fed on cases.[32] However, fifteen years of experimentation with thousands of flies and hundreds of test animals produced only four transfers by bite. Other insects, particularly fleas and bedbugs, were studied and eliminated as vectors. Other means of spread were suggested by the demonstration of parasites in nasal secretions, urine and feces of cases. It was shown that hamsters could be infected by mouth. Direct transmission may occur but is probably uncommon in view of the epidemiology of the disease. Recently new evidence has made it virtually certain that sandflies are the significant vectors. Smith *et al* maintained infected sandflies on fruit juices instead of the customary blood meals, and infections have consistently developed in hamsters and mice bitten by such flies.[33] In these flies the pharynx often becomes blocked with the simple flagellate stages, a phenomenon not observed in blood-fed flies, and in their vigorous efforts to feed the "blocked" flies apparently are more likely to inject parasites.

The sandfly vectors belong to the genus *Phlebotomus* of the family of small flies, Psychodidae. The most important species are *P. argentipes* in India, *P. chinensis* in China, and *P. major* and *P. perniciosus* in the Mediterranean region.[33a] The vectors in other areas are as yet unknown. The adults are minute, night-biting insects. They fly weakly and travel very short distances but penetrate ordinary window-screening with ease. The larvae develop in loose damp soil or debris, mostly in cracks in walls, cliffs, caves, etc., the entire life cycle requiring one to two months.

Control of the transmitting insects is difficult, though some success has resulted from cleaning up potential breeding places near human habitation. Fine-mesh bed nets, fans and repellents are of value as protection against the bites. Large-scale treatment of human cases is widely used for control.

L. Tropica.—This parasite was discovered by Wright,[34] in 1903, in an Armenian patient in Boston. Morphologically and culturally identical

[32] Knowles, Napier and Smith: Ind. Med. Gaz., 1924, *59*:593.

[33] Smith, Halder and Ahmed: Ind. Jour. Med. Research, 1940, *28*:575; *ibid.* 1941, *29*:783, 799.

[33a] The name *Phlebotomus* is widely used but is probably incorrect. Many authors now use *Flebotomus*.

[34] Wright: Jour. Med. Res., 1903, *10*:472.

with *L. donovani*, it differs in infecting primarily the skin, where it proliferates in the macrophages of the subcutaneous tissue. In man, dogs and wild rodents it produces large single or multiple ulcers, usually of exposed parts of the skin, known as oriental sores. These lesions appear after an incubation period of ten days to several months. They increase to a diameter of 1 to 3 cm. and heal spontaneously after several months to leave a disfiguring scar. Permanent immunity follows infection, and deliberate induction of sores on unexposed parts of the body has long been practised in the Middle East to avoid the disfigurement of exposed scars. Transfer by direct contact, fomites and house flies is possible, but the principal method of transmission is undoubtedly by sandflies in a manner similar to kala-azar. Oriental sore occurs in Southern Asia, Southern Russia, the Near East, Equatorial Africa and the Mediterranean region, the principal vectors being *P. sergenti* and *P. papatasii*. Laboratory diagnosis is made by identification of the parasites in stained smears of scrapings from the lesions. Local or systemic antimony treatment is specific.

L. Braziliensis.—This parasite resembles *L. tropica* but often shows a predilection for mucocutaneous borders, as around the mouth and nose. Here it produces an eroding lesion, which lasts for months. Extension to the pharynx may result in death. The disease, known by various names, of which uta and espundia are the most common, has a wide distribution in South America, extending to Southern Mexico.

SPOROZOA

Malarial Parasites.—With the exception of a rare intestinal parasite, the only sporozoa infecting man are the malarial parasites. They were first recognized by Laveran[35] in 1880, and the life-cycle in human erythrocytes was described by Golgi.[36] Manson's suggestions led Ross[37] to the demonstration in 1898 of mosquito transmission of avian malaria, and Grassi, Bignami and Bastianelli[38] later in the same year showed the mechanism of spread of human malaria. Man harbors three or possibly four species of malarial parasites, of which *Plasmodium vivax* will serve as an example.[39]

Characteristics and Life-Cycle.—In fresh preparations of infected blood the parasites of *P. vivax* appear as clear areas in the erythrocytes. They contain yellow or brown granules of pigment, a digestion product of hemoglobin which has been identified microchemically as hematin. In direct blood films colored with stains such as Giemsa the parasites show blue cytoplasm and violet-red nuclei (Fig. 199). The earliest stage in the erythrocyte, the *ring* stage, consists of a thin ring of cytoplasm with a nucleus at one side. The parasite grows, becoming an irregular uninucleate body containing several brown pigment granules. This stage is known as the *ameboid trophozoite*. The parasitized cell has now enlarged somewhat and

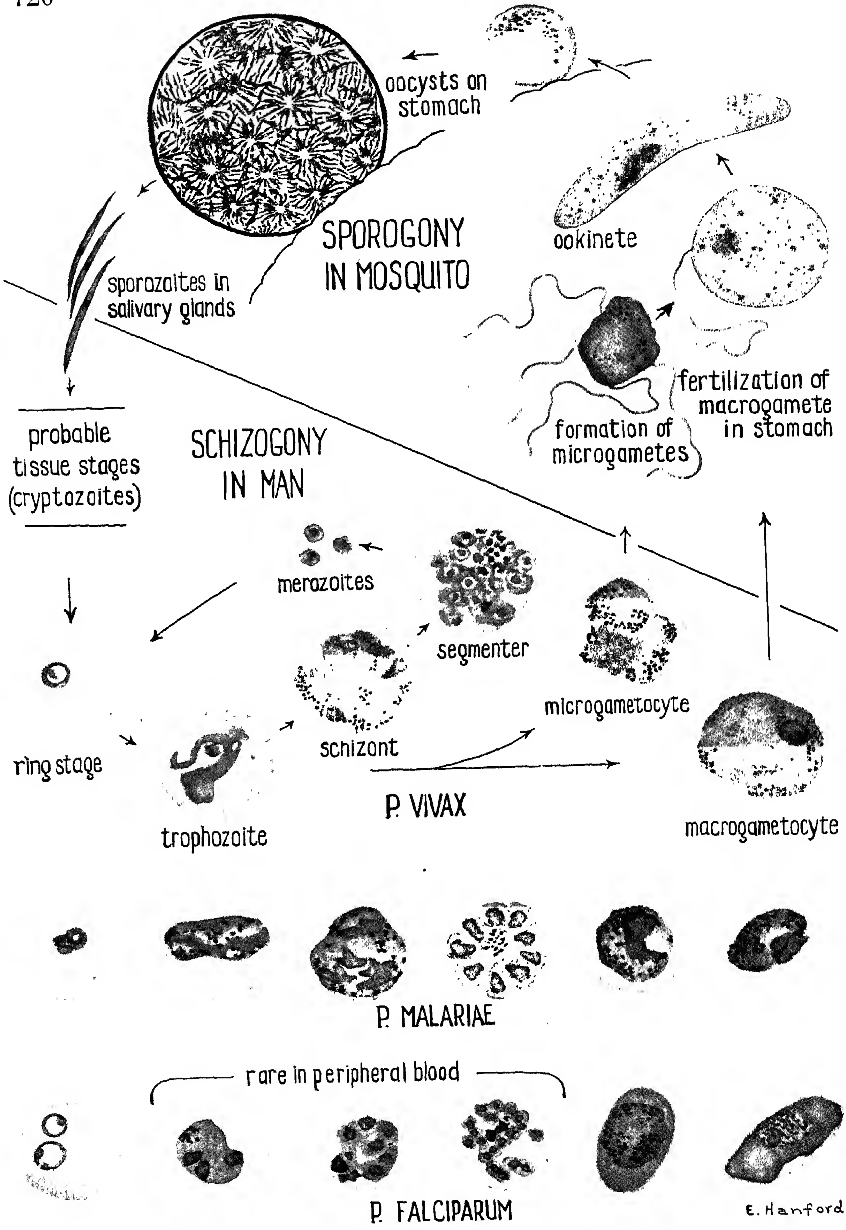
[35] Laveran: Bull. Acad. Med., 1880, *9:*1235, 1268, 1346.

[36] Golgi: Arch. Sci. Med., 1889, *13:*173.

[37] Ross: Ind. Med. Gaz., 1898, *33:*401, 448.

[38] Grassi, Bignami and Bastianelli: Atti. R. Accad. Lincei Rendic. Ser. 5, 1898, *8:*21; *ibid.*, 1899, *8:*100, 434.

[39] An excellent comprehensive book is: *Human Malaria*, A.A.A.S. Pub. No. 15. Washington. 1941.



oocysts on stomach
ookinete
SPOROGONY IN MOSQUITO
sporozoites in salivary glands
fertilization of macrogamete in stomach
formation of microgametes
probable tissue stages (cryptozoites)
SCHIZOGONY IN MAN
merozoites
segmenter
microgametocyte
ring stage
trophozoite
schizont
P. VIVAX
macrogametocyte
P. MALARIAE
rare in peripheral blood
P. FALCIPARUM
E. Hanford

Fig. 199.

may show scattered throughout its cytoplasm minute red granules, "Schüffner's dots," which are apparently a result of injury to the cell. The parasite continues to grow, accumulating more pigment. Eventually it nearly fills the erythrocyte, which is now about one and one half times its normal diameter. The nucleus divides repeatedly until twelve to twenty four, usually about sixteen, nuclei are present. This is the *schizont* stage. Finally, in the *segmenter* stage, the cytoplasm divides, a portion surrounding each nucleus. The pigment is left in a dense clump, and the cell disintegrates to release the daughter cells, or *merozoites*, into the plasma. Here they invade fresh erythrocytes and repeat the cycle. The above process of growth and multiple fission is known as *schizogony*. It occupies, in *P. vivax*, about forty-eight hours, and the growth is regulated by the daily cycle of activity of the host, so that segmentation usually occurs at about the same time every other day.

After several schizogonic cycles a difference may be noted in the infection. Some ameboid stages, instead of becoming schizonts and undergoing asexual reproduction, develop into large uninucleate parasites with scattered pigment granules. These are the sexual stages. The female, or *macrogametocyte*, shows a compact, dark red nucleus and intense blue cytoplasm. The male, or *microgametocyte*, has a more diffuse, less deeply-stained nucleus and the cytoplasm is paler, often pinkish rather than blue. The gametocytes undergo no further development in man, eventually degenerating or being destroyed unless they are taken up by a susceptible mosquito.

In the stomach of the mosquito, gametes are produced. The macrogametocyte escapes from the erythrocyte and becomes a single *macrogamete*, corresponding to a metazoan ovum. The microgametocyte produces at its surface, by a process generally called "exflagellation," four to eight long, whip-like *microgametes*, counterparts of the spermatozoa of higher animals. One of these actively wriggling microgametes fertilizes a macrogamete. The resulting zygote elongates, becoming a motile *ookinete* about 20 μ long. The above process in the mosquito occupies one to two days. Many ookinetes are destroyed in the mosquito stomach, but some penetrate its wall and come to rest as round *oocysts* on the outside of the stomach. Here they grow, undergoing nuclear multiplication, until a diameter of about 50 μ is attained, the oocyst now containing many hundreds of nuclei. Each acquires a bit of cytoplasm and becomes a spindle-shaped body, about 8 μ long, the *sporozoite*. With the rupture of the oocyst, these sporozoites scatter throughout the body of the mosquito. Many accumulate in the salivary glands, where they are injected into man by the biting mosquito. The complete development in the mosquito requires from one to two weeks. 25° C. is said to be the optimal temperature, no development occurring below 15° C. nor above 30° C.

The early development of the parasite in the body of man has not been elucidated. Schaudinn,[39a] in 1902, described penetration of the sporozoite

[39a] Schaudinn: Arb. a. d. Gesundheitsamte, 1902, *19*:169.

Fig. 199.—Life cycle and comparative morphology of the malarial parasites of man. (Schizogonic stages redrawn from Huff, *Manual of Medical Parasitology*). Oooysts × 600; remaining stages × 2000.

into an erythrocyte and initiation of schizogony, but recent evidence casts serious doubt on this report. First, many investigators have failed to confirm Schaudinn's observation. Second, it has not been possible to infect normal individuals by the transfer of large quantities of blood drawn from infected persons during the first few days of the incubation period. Third, drugs highly effective against the parasites in erythrocytes have no effect during the early incubation period. These facts suggest that during the incubation period the parasites are unlike those later seen in the erythrocytes and are not present in the peripheral blood. Search for parasites during the early incubation period in man has resulted only in dubious findings.

In a species of chicken malaria, however, recent investigation has shown the steps from inoculation of sporozoites to infection of the erythrocytes.[40] Sporozoites inoculated into the skin soon enter macrophages of the subcutaneous tissue. Here, known as *cryptozoites*, they undergo a type of schizogony fundamentally like that in erythrocytes except that no pigment is produced (Fig. 200). In about thirty-six hours segmentation occurs, producing merozoites which invade new macrophages and repeat the cycle.

Fig. 200.—Cryptozoites of a chicken malarial parasite. (Courtesy of Huff and Coulston. Drawn by Esther Bohlman.)

Some of the second generation merozoites enter erythrocytes to establish the peripheral blood schizogony. Others continue to reproduce in tissue cells as *exoerythrocytic* stages. Since their discovery by Raffaele in 1934 and 1936 and Huff and Bloom in 1935, the exoerythrocytic stages of avian malarias have received much attention.[41] Neither exoerythrocytic schizonts nor cryptozoites have been definitely observed in man, but the indirect evidence strongly suggests that human malarial sporozoites probably develop into cryptozoites, which eventually give rise to the classical erythrocytic parasites in the peripheral blood.

In drawn blood with added dextrose[42] malarial parasites persist for a few days and may multiply to some extent. This is not of practical value, and cultivation in the absence of erythrocytes has not been reported.

The life-cycles and morphology of the other human malarial parasites in erythrocytes are fundamentally similar to those of *P. vivax* (Fig. 199). *P. malariae* requires seventy-two hours for the completion of schizogony.

[40] Huff and Coulston: Jour. Infect. Dis., 1944, *75*:231.
[41] *Cf.* Porter and Huff: Amer. Jour. Trop. Med., 1940, *20*:869.
[42] Bass and Johns: Jour. Exp. Med., 1912, *16*:567.

The erythrocytic parasites show several differences of diagnostic value. No granules of the type of Schüffner's dots are seen in the infected cells. The cells are not enlarged during growth of the parasites. The older trophozoite is not ameboid like that of *P. vivax* but commonly exhibits a "band" form across the parasitized cell. The segmenter produces six to fourteen, usually eight, merozoites, arranged in a "rosette" form. The gametocytes are small and the pigment typically occurs in abundant, large dark granules. The optimal temperature for sporogony in the mosquito is said to be about 22° C., and sporogony is slow, requiring three weeks or more.

P. falciparum, like *P. malariae*, causes no enlargement of the infected cells, but some parasitized cells show granules, Maurer's dots, which correspond to the Schüffner's dots of *P. vivax*. Schizogony results in six to twenty-four merozoites. The schizonts and segmenters typically accumulate in capillaries of internal organs, appearing in the peripheral blood only in very heavy infections. Normally, therefore, only ring stages and gametocytes are seen in blood films. Infection of erythrocytes by two or more rings is common and the rings often contain two nuclei. The gametocytes are distinctive, exhibiting a characteristic sausage shape which distorts or obliterates the infected cell. Because of their shape, they are commonly called "crescents." The pigment is finely granular or amorphous in appearance, and in the gametocytes it is typically concentrated about the nucleus. The optimal temperature for sporogony is said to be 29° C.

P. ovale is a rare species showing similarities to both *P. vivax* and *P. malariae* but characterized chiefly by the fact that the infected cell is often distorted into an oval. Its significance is not known, although many investigators consider it an aberrant type of *P. vivax*.

Malaria.—Vivax malaria, or *benign tertian malaria*, is featured by typical chills and fever occurring at the time of segmentation of the peripheral blood parasites. These paroxysms begin with an acute, shaking chill while the temperature is rising. A "hot stage" occurs at the fever peak, the patient feeling unbearably hot and the oral temperature usually reaching 104 or 105° F. This gives way to a "sweating stage" during which the fever falls rapidly to normal or slightly below. Parasite products or red blood cell contents released at disruption of the infected cells are presumably responsible for the paroxysm. Paroxysms coincide, as stated above, with the time of parasite segmentation. Hence they occur every forty-eight hours if all the parasites reproduce on the same day. Often, however, distinct broods of parasites segment on alternate days. Vivax malaria commonly shows quotidian (daily) chills for several days followed by the suppression of one brood of parasites with resultant tertian chills (every other day). Between paroxysms the patient feels and appears normal.

Significant anemia occurs commonly in vivax malaria but is rarely serious unless other factors are involved. A week or more after the beginning of an attack, particularly in children, the spleen usually enlarges and remains enlarged for two to six months after termination of the attack. The untreated attack typically lasts three to six weeks, often with temporary cessations of clinical activity. It is followed by a period of latency,

which probably lasts two to three years as a rule, during which parasites cannot be found microscopically in the peripheral blood. That they are present, though in small numbers, is attested by the fact that the blood is infective if inoculated into normal individuals. In about one-third of cases renewal of clinical activity, or relapse, occurs during this period, the clinical picture resembling that in the primary attack.

Malaria due to *P. malariae* is known, because of the seventy-two hour cycle of reproduction, as *quartan malaria*, the chills occurring every third day. It is basically similar to vivax malaria, though the paroxysms are often more severe. The incubation period is usually long, three weeks or more, and the period of clinical activity typically lasts for several months. Relapses are uncommon, but latent infection may persist for many years, as shown by the occasional development of quartan malaria in recipients of blood transfusions from persons who have not shown evidence of infection for thirty years or more.

Falciparum malaria is widely known as *malignant tertian* or *estivo-autumnal malaria*. As noted above, *P. falciparum* is characterized by accumulation of schizogonic stages in internal organs. As a result, in addition to or instead of the typical paroxysms, which are quotidian or tertian, various local manifestations of the disease may be prominent. This is especially true in the tropics where such "pernicious" malaria may follow a series of typical chills. The most common types are algid (cold) malaria, gastrointestinal manifestations, and cerebral malaria with coma and often death. These peculiarities of falciparum malaria make it the cause of most of the deaths attributable to human malarial infection and an important factor in many deaths traced to other diseases. Blackwater fever, a dangerous hemoglobinuria, is associated with falciparum malaria and is thought to be an occasional late effect of repeated infection. *P. falciparum* infections relapse uncommonly and rarely persist more than a year or two.

Immunity.—Malaria exhibits what has been termed "infection immunity." After recovery from an acute attack, the individual is highly resistant to superinfection with a strain similar to that already present in his body, but the resistance lasts for at most a few months after elimination of parasites from the body. This immunity depends on active phagocytosis and digestion of parasitized erythrocytes by the macrophages of spleen, liver and bone marrow.[43] That the parasitized cells are sensitized by antibodies is suggested by the fact that serum from recovered animals partially protects against infection.[44] The immunity to superinfection is species- and strain-specific. The latent malarial infection is a state of balance between reproduction of the parasites and their destruction by the body defenses. Relapse occurs when this balance is disturbed, permitting the parasites to increase greatly in numbers. The factors are little known, though it has recently been shown that operations increasing blood sugar induce relapse in avian malaria.[45]

Partial inhibition of avian and monkey malarial infections has been

[43] *Cf.* Taliaferro: *Human Malaria*, p. 239.
[44] Coggeshall and Kumm: Jour. Exp. Med., 1937, *66*:177.
[45] Gajewsky and Tatum: Jour. Infect. Dis., 1944, *74*:85.

achieved with killed vaccines. There is no evidence concerning human malaria, but, because of the nature of immunity to malaria, it seems unlikely that vaccination would be of practical value. Serum agglutination of parasitized cells or isolated parasites has been reported in experimental infections. It is species-specific. Complement-fixing antibodies occur in the serum and are group-specific, reacting with extracts of avian and simian malarial parasites as well as with those from man. Recent data indicate that this reaction may be of value in the diagnosis of latent human malarial infections.

DIAGNOSIS.—Laboratory diagnosis of acute malaria rests on the finding of parasites in peripheral blood films. They are most easily identified in thin films stained with Giemsa, but, since parasites are often scanty in the peripheral blood, dehemoglobinized thick films, similarly stained, are widely used. The rapid staining method of Field[46] is of especial value for such preparations. The fact that parasite morphology is abnormal in thick films is offset by the much greater volume of blood which can be examined in a comparable time. In surveys the characteristic spleen enlargement is a nonspecific but highly suggestive indication of current or recent malarial infection. Because it usually persists for several months it affords a more stable index of the infection rate in an area than do clinical or parasitological surveys.

CHEMOTHERAPY.—Treatment of malaria has for centuries depended on the bark or extracts of the bark of cinchona trees. The best known alkaloid is quinine, though others are effective against the parasites. Quinine neither prevents natural infection nor eradicates parasites from the body, but is of great clinical value because it rapidly reduces the number of blood parasites below the density necessary to produce symptoms. It is active against all blood parasites except the gametocytes of *P. falciparum*. Intensive research by German industrial chemists has resulted in two significant drugs. Plasmochin, while dangerous to man in curative doses, destroys *P. falciparum* gametocytes when given in small doses and has been used in an effort to reduce mosquito infection rates. Atabrine has an action similar to that of quinine. In addition, recent evidence indicates that it can completely eliminate *P. falciparum* parasites from the body. No known drug has this property in *P. vivax* and *P. malariae* infections. Truly prophylactic drugs are unknown. However, atabrine or quinine taken continuously during exposure acts as a suppressive, holding the infection down to sub-clinical levels.

Epidemiology and Control.—Malaria is the most common infectious disease of man, occurring throughout the warmer regions of the world and extending well into the temperate zones to occupy a large part of the land area between 60° N. and 40° S. latitude. The principal factor in distribution of the infection is climate, which affects both the distribution and abundance of the mosquito hosts and the development of the parasite in the mosquito. The human malarial parasites are not found in lower animals. Man is, therefore, the only reservoir of infection. Racial immunity is known in the case of *P. vivax*. Negroes are less readily infected than whites

[46] Field: Trans. Roy. Soc. Trop. Med. and Hyg., 1940, *33*:635, *34*:195.

and seldom show symptoms. Local populations with high natural immunity to *P. falciparum* have also been reported.[47] Age resistance is not known, but in endemic regions clinical malaria may be rare in native adults who have been constantly reinfected with the local strains of parasites.

Barring rare congenital cases, blood transfusions and accidental transfers of blood, as by drug addicts sharing contaminated syringes, human malaria is transmitted exclusively by the bites of certain mosquitoes of the genus *Anopheles*. This genus, comprising some two hundred species

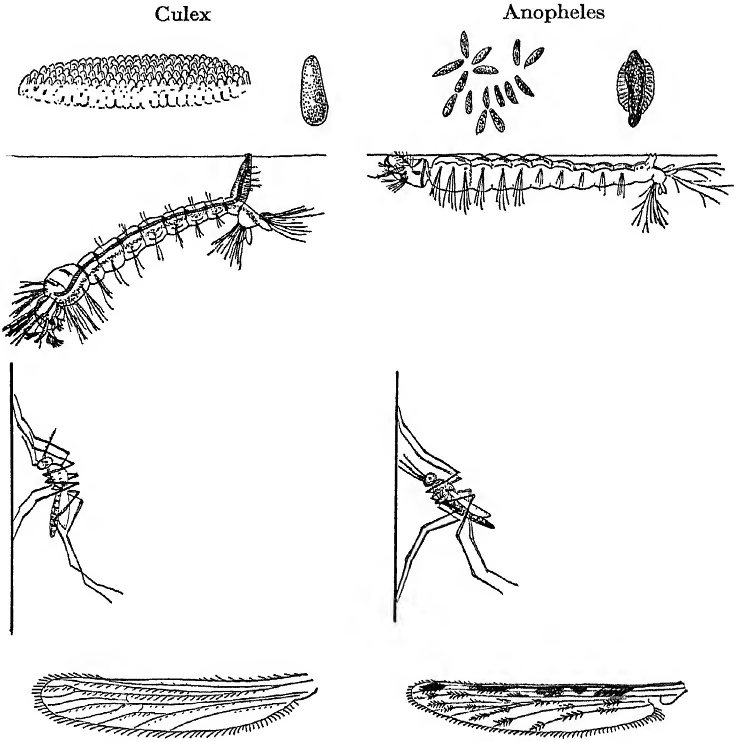


Fig. 201.—Comparison of *Culex* and *Anopheles*. Eggs, larvae (note position), position of insects at rest, wings (Kolle and Hetsch, partially redrawn).

throughout the world, is readily distinguished from the common house mosquitoes of the genera *Culex* and *Aedes* by several characteristics (Fig. 201). The adult generally rests at an angle to the surface, with proboscis, head and body in a straight line, whereas the others rest parallel, with the head and proboscis turned down. The wings are usually spotted, those of other mosquitoes being unmarked. The palps of the female are as long as the proboscis, giving the impression of three long appendages from the

[47] Swellengrebel: Proc. Third Int. Cong. Microbiol., 1940, p. 119.

head in addition to the antennae, whereas the other genera have short, barely noticeable palps. The eggs bear inflated floats and are laid singly on the water, whereas in *Culex* they are laid in rafts on the water and in *Aedes* they are deposited singly on damp surfaces. The larvae lie flat at the surface of the water, feeding on floating particles, while in the other genera they hang down in the water from an elongated breathing tube and usually feed on the bottom.

Only the female mosquitoes feed on blood, most *Anopheles* biting at dusk or in the night. The breeding places vary greatly in character, almost any type of water collection serving to support the larvae of one or more species of *Anopheles*. Most species, however, are quite specific in the types of water collections they choose. The principal malarial vector in the southeastern United States, *A. quadrimaculatus*, breeds chiefly around the debris- and weed-covered edges of swamps, ponds and sluggish streams. Important malarial vectors, however, are found in hill streams (*A. minimus* in Southern Asia), brackish marshes (*A. maculipennis* in southern Europe), small temporary pools (*A. gambiae* in Africa), water held in plants growing in the tops of trees (*A. bellator* in Trinidad), etc.

Not all species of *Anopheles* serve equally efficiently as vectors of malaria, about seventy-five being considered dangerous carriers in one or more regions.[48] The chief determining factors are abundance, contact with man and susceptibility. The last varies greatly among species and among populations within species. Little is known of the factors in human malaria, but simple genetic differences have been shown to determine susceptibility of some *Culex* mosquitoes to avian malaria.[49] In most situations which have been adequately studied, contact with man is the principal factor in the importance of species of *Anopheles*. Several susceptible species are abundant in the southeastern United States, but in most of this area only one, *A. quadrimaculatus*, shows sufficient preference for human blood to serve as an important carrier. In Europe races[50] of *A. maculipennis*, distinguishable only by egg patterns, differ so greatly in their relative preferences for human and animal blood that some are dangerous vectors while others, equally susceptible, are insignificant in the spread of malaria.[51]

Since prophylactic or universally curative drugs are not available, malaria control rests on the interruption of the mosquito phase of the life-cycle. This may be accomplished by reduction in mosquito numbers or prevention of contact between mosquitoes and man. If mosquito reduction is to be attempted, the differences in relative importance of different species, or even of the same species in different regions, require that the principal vectors in a given area be determined before control is attempted. Such determination is of value in avoiding waste of resources on unimportant mosquitoes. Furthermore, unplanned control efforts may completely miss the principal vectors and have even, on occasion, made matters worse by

[48] Russell, Rozeboom, and Stone: *Keys to the Anopheline Mosquitoes of the World.* American Entomological Society. Philadelphia. 1943.

[49] Huff: Jour. Prev. Med., 1931, *5*:249.

[50] These are now designated as separate species by many authors.

[51] Hackett: *Malaria in Europe.* Oxford University Press. London. 1937.

favoring the breeding of dangerous species. The "natural index" of infection, determined by examining stomachs and salivary glands of wild-caught female mosquitoes for oocysts and sporozoites of malarial parasites, is the best guide to relative importance of a species. Unfortunately, the figure is often so low that dissection of large numbers of mosquitoes is required. In such cases expediency may justify indirect measures. Susceptibility may be determined by exposure of laboratory-reared mosquitoes to carriers and dissection of the fed females for stages of the parasite ("experimental index"). Contact with man may be measured by the location of resting mosquitoes (in houses as against stables) or by precipitin tests on fed mosquitoes to determine the type of blood they contain.

When the important species in an area have been identified, their breeding habits must be determined so that proper methods of control may be applied. *Elimination of breeding places* is the method of choice for long-range control. Water collections may be destroyed by drainage or filling. Salinity may be controlled by tide-gates. Breeding around the margins of reservoirs may be eliminated by water-level fluctuations. Shade may be increased or decreased by control of vegetation. Streams may be cleared or periodically sluiced. Specific methods, it will be obvious, depend on local conditions and the habits of the mosquitoes involved. Several effective poisons (larvicides) are available for attack on the aquatic stages. Oils sprayed on the water surface are toxic to the eggs, larvae and pupae. Paris green and the newly rediscovered DDT kill larvae which ingest them. A minor method of local value is the stocking of ponds and pools with top-feeding minnows, *Gambusia* or *Lebistes*, which eat mosquito larvae.

Attack on the adult mosquitoes is useful as a supplement to larval control or where temporary protection is necessary. Spray-killing of adults with pyrethrum in oil or especially with pyrethrum-freon aerosols is of value. Even swatting has its place. Screening, bed-nets, protective clothing, repellents and the avoidance of evening exposure outdoors all are useful and have contributed to the control of malaria.[52]

Malarial Parasites of Lower Animals.—Malarial parasites fundamentally like those of man occur in apes, monkeys, birds, bats and lizards. Since lower animal hosts are not available for the human malarial parasites, the natural parasites of monkeys and birds have been widely used experimentally. Among the important discoveries made in these infections are the mosquito transmission of malaria, the chemotherapeutic activity of atabrine, exoerythrocytic and cryptozoic stages and much of the information on immunity. The species most widely studied are *P. brasilianum*, *P. knowlesi* and *P. cynomolgi* of monkeys and *P. cathemerium*, *P. relictum*, *P. gallinaceum* and *P. lophurae* of birds.

PARASITES OF UNCERTAIN AFFINITIES

Toxoplasma.—Parasites known as *Toxoplasma* were discovered in 1908 in rodents by Nicolle and Manceaux[53] in Africa and by Splendore[53] in Brazil.

[52] Coggeshall, *et al.: War Medicine*, 1943, *3:*484, 619.
[53] *Cf.* Sabin: Jour. Amer. Med. Assn., 1941, *116:*801.

They are crescentic or oval bodies, 6 to 7 by 2 to 4 microns in size, staining blue with a red nucleus in Giemsa preparations (Fig. 202). They reproduce only in cells, chiefly monocytes and endothelial cells, dividing by binary fission, but are often found free in body fluids. Many mammals and birds are susceptible, and infection has been produced by a variety of routes, though the natural means of spread are unknown. Artificial cultivation has not been achieved. *Toxoplasma* have been reported in several cases of encephalitis in newborn infants and children,[53] and in a typhus-like pneumonitis of adults.[54] While they are commonly called sporozoa, it must be said that their relationships are unknown, and it is not even certain that they are protozoa.

Sarcocystis.—In the muscle fibers of various vertebrates, particularly ruminants and rodents, are seen long, cylindrical tubes containing numer-

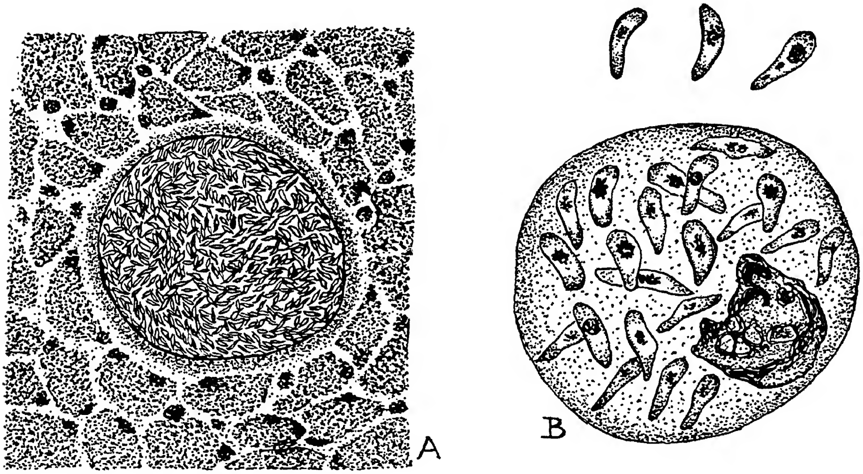


Fig. 202.—Parasites of uncertain affinities. *A*, *Sarcocystis* in transverse section of muscle, × 300 (Modified from Baraban and St. Remy). *B*, *Toxoplasma*, 3500 × (Redrawn from Chatton and Blanc).

ous crescent-shaped "spores" (Fig. 202). They are called "Miescher's tubes" for their discoverer,[55] and a number of species have been described under the generic name *Sarcocystis*. Mice become infected after ingestion of meat containing the parasites.[56] A powerful exotoxin, sarcocystin, is produced, and death sometimes occurs in heavily infected animals. Occasional human infections have been reported. Since the spores apparently result from schizogony, the group, known as *Sarcosporidia*, are commonly classed with the sporozoa, but their relationships are still insufficiently understood.

[54] Pinkerton and Henderson: Jour. Amer. Med. Assn., 1941, *116*:807.
[55] Miescher: Ber. v. d. Verhandl. Naturf. Ges. Basel, 1843, *5*:198.
[56] Smith: Jour. Exp. Med., 1901, *6*:1.

THE METAZOA

All phyla of animals other than protozoa are commonly designated by the term *Metazoa*, and it will be convenient to mention certain general characteristics of the human parasites, mostly known as "worms," which belong to this aggregate. They are usually macroscopic in size, ranging from about a millimeter to several meters in length. Their structure is generally complex, and their life cycles vary from the simple production of infective eggs or larvae to complex alternation of generations involving as many as three different hosts. An important general characteristic is that multiplication usually does not occur in the human body, so that infection is stable in the absence of reexposure. Successful cultivation of parasitic worms has not been achieved. The great variety of metazoan parasites of man makes it impossible to dicuss them extensively in a small space. Therefore, representative examples will be used wherever possible. The reader is referred to the general texts mentioned above (p. 700) for fuller discussions.

PLATYHELMINTHES

The phylum platyhelminthes, or "flatworms," is differentiated from other phyla of animals by several characteristics. They are bilaterally symmetrical and composed of the three primitive germ layers of tissues—ectoderm, entoderm and mesoderm. The body cavity is not lined with mesoderm, as in higher forms, but filled with a spongy mass of cells, the *parenchyma.* The digestive tract is absent or, if present, lacks an anus, solid wastes being regurgitated through the mouth. There are three major classes—the *Turbellaria*, which are free-living forms or external parasites of aquatic animals, exemplified by *Planaria;* the *Trematoda*, or "flukes," which are all parasitic; and the *Cestoidea*, or "tapeworms," also all parasitic.

TREMATODA

The human lung fluke, *Paragominus westermani*, and the blood fluke, *Schistosoma mansoni*, will serve as examples of this group.

Paragonimus Westermani.—The adult worm, found by Westerman in 1877 in the lungs of a tiger, was described by Kerbert[57] in 1878. Nakagawa,[58] Yokagawa,[58] and others elucidated the life-cycle. Several species have been described, but it is not certain that they are valid, and some authors believe that there is only one species, *Paragonimus westermani.*[58]

Characteristics and Life-Cycle.—The adult lung fluke is an ovoid reddish worm, 7 to 12 mm. long by 3 to 6 mm. in diameter, covered with a transparent cuticle which is studded with spines. The anatomy of a flattened specimen is shown in Fig. 203. Two muscular suckers are present, one on

[57] Kerbert: Zool. Anz., 1878, *1:*271.
[58] *Cf.* Ameel: Amer. Jour. Hyg., 1934, *19:*279.

Fig. 203.—Life-cycle and morphology of *Paragonimus.* (Larval stages redrawn from Ameel, 1934, × 120. Adult worm × 7). m, mouth; ph, pharynx; int, intestinal caecum; vit, vitellaria; ac, acetabulum; gp, genital pore; ov, ovary; sg, oötype; vdef, vas deferens; vitd, vitelline duct; t, testis; exc, excretory bladder.

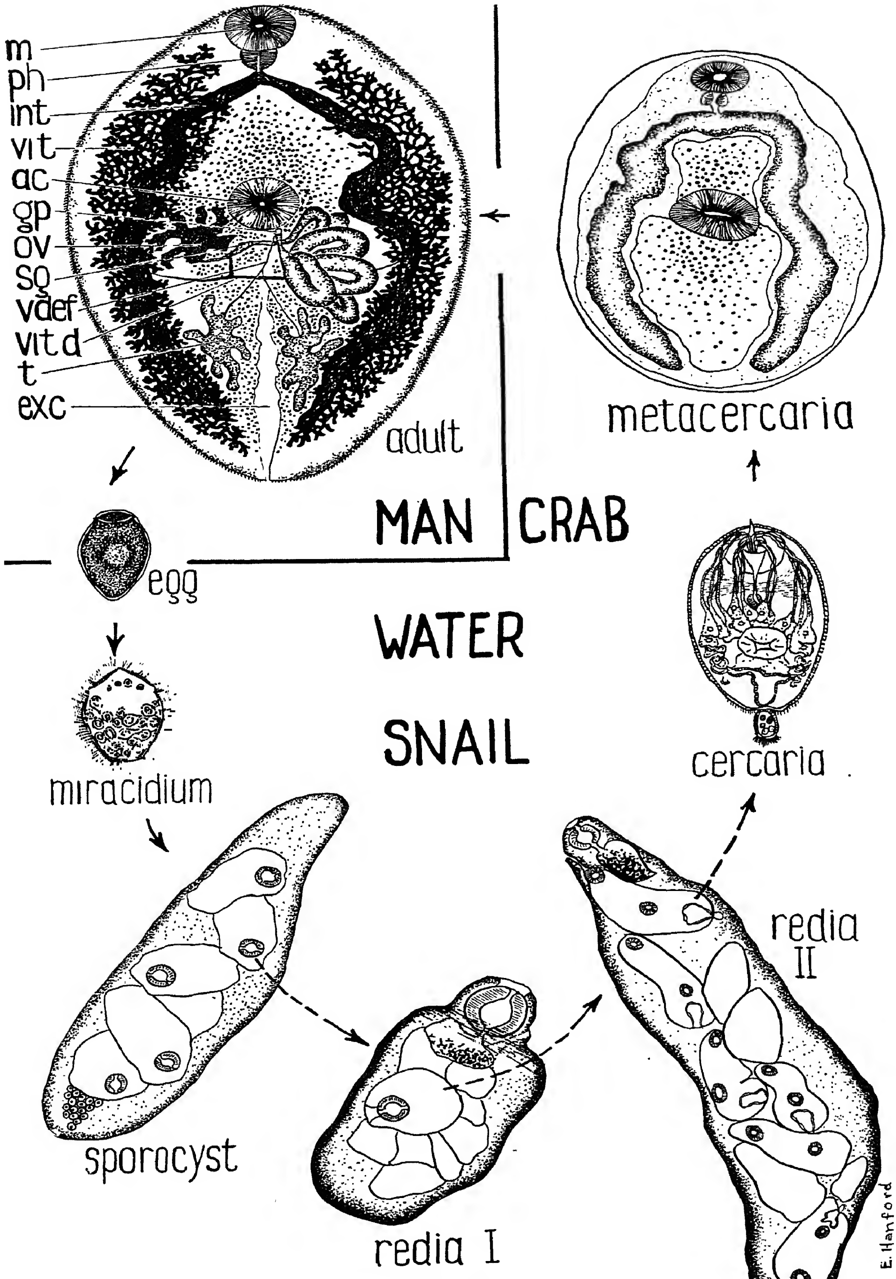

m
ph
int
vit
ac
gp
ov
sg
vdef
vitd
t
exc
adult
metacercaria
MAN
CRAB
egg
WATER
SNAIL
miracidium
cercaria
sporocyst
redia I
redia II
E. Hanford

Fig. 203.

the ventral surface, the *acetabulum*, the other at the anterior end, the *anterior sucker*, perforated by the mouth. The mouth opens into a muscular pharynx, followed by a thin esophagus. This divides into two blind *intestinal ceca* extending down the sides of the body. The nervous system is simple, and no special sense organs are present. The excretory system consists of a bladder opening at the posterior end and receiving collecting tubules which extend throughout the body, terminating in characteristic "flame cells." The circulatory system is rudimentary, consisting of indefinite channels through the parenchyma.

The adult fluke is hermaphroditic, both male and female reproductive systems occurring in the same individual. These systems are typically elaborate and, as in most flukes, are the most prominent structures of the body. In the female system a single ovary connects via an oviduct with the *oötype*, where the eggs are formed and receive their shells. In its course the oviduct receives the common *vitelline duct*, into which yolk or shell material comes from the branched *vitellaria*, or yolk glands, occupying the sides of the dorsal surface of the body. The oviduct also has a diverticulum, the *seminal receptacle*, in which sperms are stored.[59] An inconspicuous duct of unknown function, *Laurer's canal*, runs from the oviduct to an opening on the dorsal surface of the body. The oötype, surrounded by gland cells, *Mehlis' gland*, opens into the uterus, a long coiled tube which carries the completed eggs to the common genital pore near the acetabulum.

The male reproductive system consists of paired testes in the posterior part of the body, connecting by vasa efferentia with the vas deferens, which empties into the common genital pore along with the uterus. Part of the vas deferens is widened into a sperm reservoir, the seminal vesicle, and a more distal portion is the glandular *prostatic region*. The terminal portion forms a muscular copulatory organ, the *cirrus*.[60]

The eggs produced by adult worms in the lung are coughed up and either escape in the sputum or are swallowed and passed in the feces. They are ovoid, averaging 60 by 90 μ in size, and show a removable cap, the *operculum*, at one end. The embryos are undeveloped at the time of escape from the host. They develop in from two to six weeks in water, where the eggs hatch by the opening of the operculum to release a free-swimming ciliated larva, the *miracidium*. This larva survives only a few hours unless it succeeds in penetrating the tissues of a suitable intermediate host. Various snails of the genera *Melania* and *Pomatiopsis* can serve as hosts. In the lymph spaces of the snail the miracidium becomes an irregular, thin-walled sac, the *sporocyst*, growing to a final length of about 0.4 mm. Cell masses within the sporocyst enlarge, developing in about one month into twelve or more *rediae*. These escape by rupture of the sporocyst and grow to a length of about 0.3 mm. They differ from the sporocyst principally in having a rudimentary digestive tract and a *birth pore*. By a reproductive process similar to that in the sporocyst the first generation redia produces

[59] In *Paragonimus* the seminal receptacle is small, though in some human flukes it is a prominent structure.

[60] These three organs are contained in the *cirrus sac* in most forms, but this is absent in *Paragonimus*.

twelve or more second generation rediae. These escape by way of the birth pore and grow to a length of about 0.5 mm. Within each second generation redia arise twenty or more tailed larvae, or *cercariae*. The cercaria is essentially a rudimentary adult worm. It differs principally in having a small tail and two types of penetration organs, a stylet in the mouth region and several gland cells, opening at the anterior end, which secrete histolytic enzymes. About three months after entry of the miracidium the cercaria escapes from the snail and moves about in the water, dying in one to two days unless it finds a suitable second intermediate host, a crayfish or crab.

The cercaria penetrates the softer part of the integument of a crab, loses its tail and grows in the tissues into the *metacercaria*, a near-spherical body about 0.4 mm. in diameter enclosed in a cyst wall. The full development requires about one month, after which the metacercaria is infective when eaten by man. In the small intestine the cyst wall softens, releasing the metacercaria, which penetrates the wall of the small intestine and reaches the abdominal cavity within a few hours. It wanders rather aimlessly but usually passes through the diaphragm within a few days and invades the lung, where it becomes encapsulated by the tissues and grows to the adult stage. After about six weeks eggs may be found in the sputum.

Paragonimiasis.—The adult worms in the lung, and particularly the eggs they produce, cause tissue destruction, inflammation and hemorrhage. Local pneumonic processes with cough and bloody sputum are characteristic. As in most worm infections, the damage is roughly proportional to the number of organisms present. In severe cases weakness and even death may result from the extensive lung injury. Aberrant worms in other tissues, as in the brain, cause local effects.

There is no evidence concerning acquired *immunity* to infection. Human infections have been shown to persist at least six years in the absence of reexposure, but it is said that they often clear up after several years.

Diagnosis of lung fluke infection depends on the finding and identification of characteristic eggs in the sputum or feces. As in other trematode infections, the size and structure of the eggs (see Fig. 203) are diagnostic. Other trematode eggs may occur in the feces, but only those of *Paragonimus* normally occur in the sputum.

Epidemiology and Control.—Various mammals are susceptible to *Paragonimus* infection. Cats, dogs, mink, muskrats and man are the usual natural hosts. Infection in lower animals is known in Asia, Africa, North and South America, and the United States. Human infection is limited by food habits, occurring commonly only in the Far East. The metacercariae in infected crustacea are destroyed only by thorough cooking or considerable exposure to pickling sauces, and in the endemic areas fresh water crabs, raw or slightly pickled, are considered a delicacy. It should be emphasized that the asexual reproduction in snails is obligate and that only the metacercariae and possibly the cercariae are infective for mammals. Thus, direct transfer from mammal to mammal does not occur, and numerical increase of an individual infection is unknown in the absence of reexposure.

Chemotherapy of human infections is useless for control both because of its unreliability and because lower animals are an important reservoir. Destruction of snails has generally failed. The only effective control measure known is education to the dangers of eating improperly cooked fresh-water crustacea. It should be noted here that while such modification of food-habits is the best control method for all human trematode infections except the blood flukes, established habits and prejudices often interpose difficulties in its execution.

Fasciolopsis Buski.—The large adults of this species attain a length of 7 cm. (Fig. 204). They are found attached to the mucosa of the small intestine of man and the pig. The infection is most common in China but

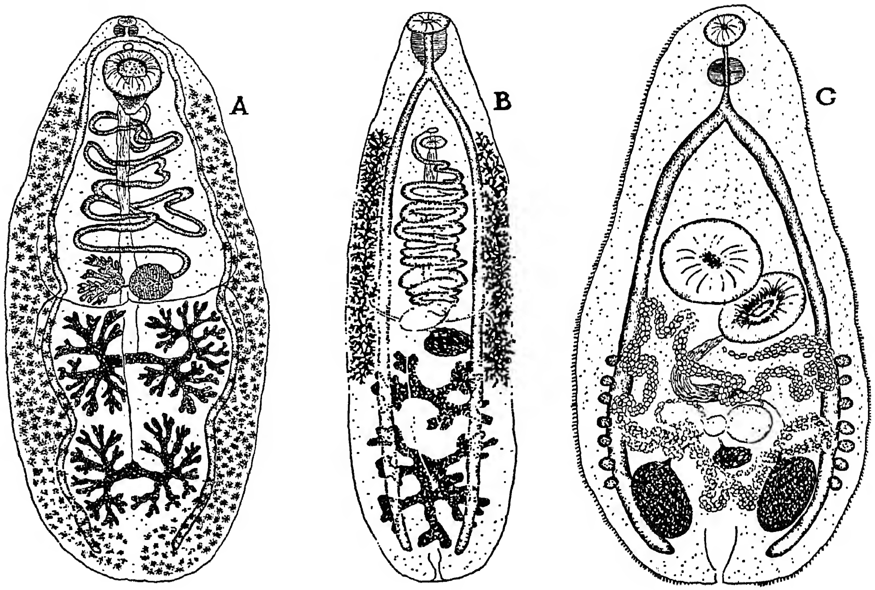


Fig. 204.—Trematode parasites of man. *A*, *Fasciolopsis buski;* × 3. *B*, *Clonorchis sinensis;* × 5. *C*, *Heterophyes heterophyes;* × 60. (Adapted from various sources.)

occurs in other parts of Asia. The large eggs (average 140 by 80 μ, see Fig. 205) escape in the feces and develop in water, where the miracidia invade snails of the genera *Segmentina* and *Planorbis*. The development in the snail, like that of *Paragonimus*, comprises a sporocyst generation and two generations of rediae. The long-tailed cercariae encyst on aquatic plants, particularly the water chestnut, as metacercariae. These plants eaten raw convey the infection to man or the pig. Symptoms are generally related to the intensity of infection. They consist of intestinal disturbances and generalized edema, which has been attributed to toxemia but may be merely nutritional, resulting from the food-robbing of the host by the worms. Severe infections may cause death. As in many other intestinal

worm infections, β-naphthol and carbon tetrachloride are efficient drugs. Control is achieved by treatment of human infections, proper sewage disposal, and education to the dangers of eating raw plants from contaminated water. The problem is complicated by the practice, common in endemic areas, of using human feces as fertilizer.

The closely related *Fasciola hepatica* infects the livers of sheep and cattle, causing "liver rot," and is of great economic importance. It is important also because it was the first trematode whose life cycle was worked out. Occasional human cases are known. Another economically important parasite of the liver of herbivores, *Dicrocoelium dendriticum*, has been found rarely in man.

Heterophyes Heterophyes.—*Heterophyes heterophyes* is a small trematode, about 1.5 mm. long, of the intestine of man, cats, dogs and other fish-eating mammals in Egypt, Asia Minor and Asia. The eggs are small,

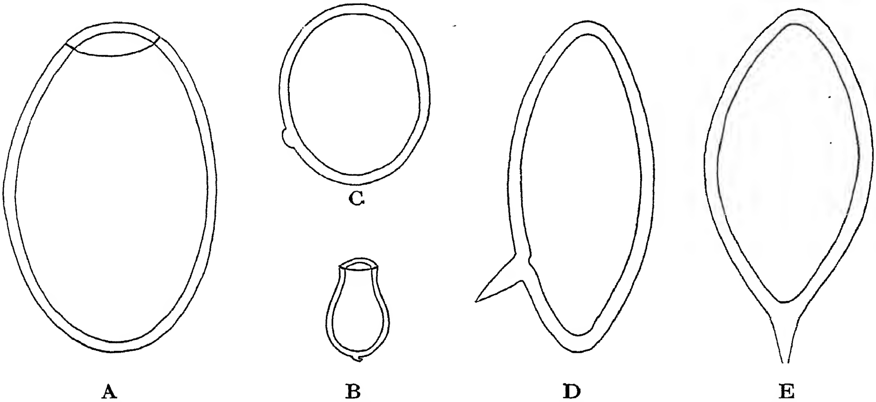


Fig. 205.—Shells of some trematode eggs; × 300. *A, Fasciolopsis buski. B, Clonorchis sinensis* (*Heterophyes heterophyes* is very similar). *C, Schistosoma japonicum. D, S. mansoni. E, S. haematobium.*

averaging 25 by 16 μ (Fig. 205). Metacercariae are ingested by man in insufficiently cooked or salted fish. The adult trematodes produce only minor gastro-intestinal disturbances, but it is reported that the eggs are often deposited deep in the mucosa, whence they reach the general circulation and are localized in various distant tissues.[61] The inflammation in these tissues, particularly the myocardium and brain, is said occasionally to cause serious symptoms or death. Several related species attack man. The most important of these is *Metagonimus yokagawai*, which occurs in man and other fish-eating animals in the Balkans, Palestine and Asia.

Other Intestinal Trematodes.—Several species of *Echinostoma*, *Echinochasmus* and related genera occasionally parasitize man. They are elongate trematodes with a ring of large spines on the head region. The human infection is apparently acquired by ingestion of raw snails containing the metacercariae. Various mammals and birds are the normal hosts

[61] Africa, DeLeon and Garcia: Acta Med. Philippina, Monogr. Ser., 1940, *1*.

of the adult worms. Occasional human infections are also reported with *Gastrodiscoides hominis* and *Watsonius watsoni*. Their life cycles are unknown, but since they chiefly parasitize herbivorous mammals it is presumed that the metacercariae occur on plants.

Clonorchis Sinensis.—The Chinese liver fluke, *Clonorchis sinensis*, is a thin, elongated trematode, 1 to 2 cm. in length. The adult worm inhabits the smaller bile ducts of man, cats, dogs and other fish-eating mammals in China and Japan. The eggs, averaging 29 by 17 μ, pass out with the bile and escape in the feces. They are fully developed but do not hatch in the water. Upon ingestion by suitable snails, usually of the genera *Parafossalurus* and *Bithynia*, they hatch and the miracidia become sporocytes in the lymph spaces. Rediae produced in the sporocyst give rise to long-tailed cercariae. These encyst as metacercariae in the tissues and on the skin and scales of various fresh-water fish. Ingested metacercariae hatch in the small intestine, and the young worms migrate up the bile ducts to develop into adults three to four weeks later. Thorough cooking (100° C. for fifteen minutes) is necessary to destroy the metacercariae. Infection of man occurs by ingestion of the metacercariae, possibly in drinking water or by contamination of the fingers in handling infected fish, but usually by consumption of insufficiently pickled or cooked fish, a favorite article of diet in the endemic regions. Hazards are increased in these areas by the practice of propagating fish for market in ponds fertilized with human feces.

Human infection is characterized by proliferation of the bile-duct epithelium and of the surrounding connective tissue. This results in liver cirrhosis, destroying liver parenchyma and obstructing portal blood flow. Intestinal disturbances, liver enlargement and ascites are the common symptoms, severe infections resulting in death. No reliable drugs are known. Control, as in the trematode infections discussed above, depends principally on education concerning avoidance of undercooked infected food, in this case fresh-water fish.

Related species, particularly *Opisthorchis felineus* and *O. viverrini*, infect fish-eating mammals and, less commonly, man in parts of Central Europe and Asia.

The Human Blood Flukes.—Bilharz[62] found trematodes in the mesenteric veins of an Egyptian patient. Three species are now known to cause schistosomiasis or, as it is called in much of the medical literature, "bilharziasis" in man. *Schistosoma mansoni* is taken here as an example.

Morphology and Life-Cycle.—Unlike the trematodes discussed above, the adults of *Schistosoma mansoni* are unisexual, male and female reproductive systems occurring in separate individual worms. The adults are found together in the smaller mesenteric veins, the long, thin female enclosed in a groove on the ventral side of the male (Fig. 206). The cylindrical female is 1 to 1.5 cm. in length by about 0.25 mm. in diameter. An anterior sucker surrounds the mouth and shortly behind it is a stalked acetabulum. The intestinal ceca, unlike those of *Paragonimus*, unite before the middle of the body, a single tube continuing to the posterior end. The oval ovary lies immediately anterior to the union of the intestinal ceca, and the uterus,

[62] Bilharz: Zeits. f. Wiss. Zool., 1852, *4*:72.

containing eggs, extends forward to open behind the acetabulum. The posterior half of the body is occupied by vitellaria. The male is about 1 cm. long by about 1 mm. in diameter, its integument covered with coarse tubercles. The suckers and alimentary tract are similar to those of the female. The reproductive system consists of a cluster of eight or nine round testes in the anterior region emptying into a seminal vesicle, which opens to the outside just behind the ventral sucker.

The large eggs, averaging 150 by 65 μ, have no operculum but show a prominent spine at the side (Fig. 205). They are deposited in the fine venules of the mesenteric veins in the wall of the intestine, where they lodge, held by the spine in the distended vessels. Stasis of the blood, injury

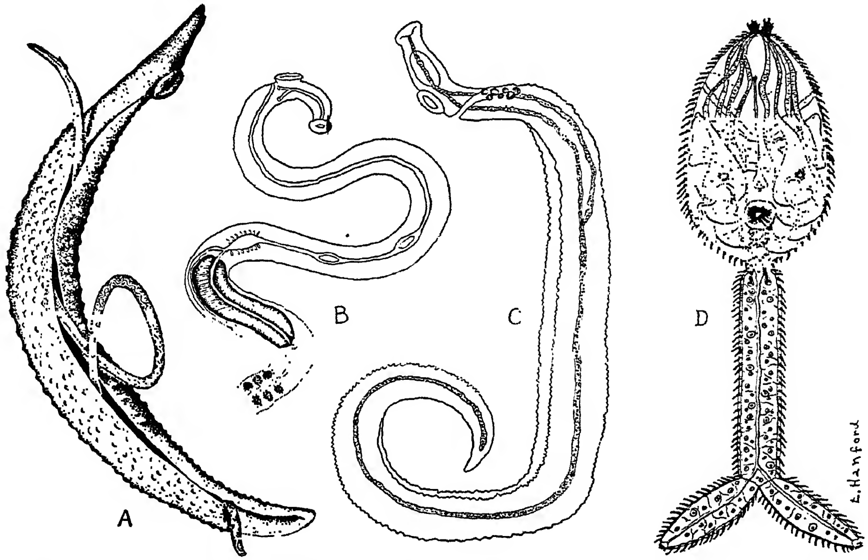


Fig. 206.—*Schistosoma mansoni. A*, Adult worms; × 8. *B*, Anterior end of female worm; × 20. *C*, Adult male; × 10. *D*, Cercaria; × 180. (*A* and *C* redrawn from Manson-Bahr. *D* redrawn from Cort.)

by the spine and enzymes secreted by the developing embryo combine to rupture the vessel, allowing the eggs to escape into the tissues and eventually into the lumen of the intestine. They are mature at the time they leave the body in the feces. On dilution of the feces with water, the shell ruptures, releasing the miracidium. In suitable snails of the genera *Planorbis* and *Physopsis* there are two generations of sporocysts resulting, after about six weeks, in the production of cercariae with long, forked tails (Fig. 206). Escaping into the water the cercariae swim actively or rest at the surface, dying in about three days if they do not reach a susceptible final host. Coming in contact with human skin they attach and, as the surface film of water is drying, actively penetrate the skin. The tail is lost and the parasite is carried via lymph and blood to the portal veins in the liver. Here

maturation occurs to the adult forms, which mate and migrate distally in the portal system to the mesenteric veins. Four to eight weeks after infection the sexually mature worms begin egg-production. The infection may persist for many years without reexposure.

The other species of human blood flukes differ from *Schistosoma mansoni* in size, details of morphology, egg structure, location in the definitive hosts, types of snail hosts and geographical distribution. *Schistosoma haematobium* adults occur predominantly in the venules of the urinary bladder wall, the eggs escaping in the urine. They are large, 150 by 60 μ, with a terminal spine. The male, 1 to 1.5 cm. long, is covered with fine tuberculations and has four large testes. The female is about 2 cm. long. Snails of the genera *Planorbis*, *Physopsis* and *Bulinus* serve as intermediate hosts.

Schistosoma japonicum occurs in the venules of the intestine. The eggs average 80 by 65 μ, with a small lateral knob rather than a spine. They escape in the feces, the miracidium invading snails of the genera *Blanfordia* and *Hemibia*. The male is 1 to 2 cm. long, with a smooth integument, and has seven or eight testes. The female is 1 to 2.5 cm. long.

Schistosomiasis.—Penetration of the skin by the cercariae may cause a mild dermatitis. In the case of the human blood flukes this is not so prominent as in that of several species normally parasitic in lower animals. In parts of the northern United States and of Europe, these species cause a severe schistosome dermatitis or "swimmer's itch." There is no evidence that these worms mature in man.

The important disease processes of human schistosomiasis are the result of tissue destruction by the eggs. In the case of *Schistosoma mansoni* this results in dysentery and later in benign tumors, constrictions or fistulae of the lower intestine. Eggs carried to the liver arouse inflammation with enlargement and cirrhosis resulting in ascites. Enlargement of the spleen is common. *Schistosoma japonicum* produces a similar picture, the liver pathology generally predominating. In infection with *Schistosoma haematobium* the worms and eggs in the wall of the bladder cause hematuria, pain, chronic inflammation, calculi, benign tumors and fistulae. Severe cases show grave injury to the entire urinary tract. In all blood fluke infections the benign tumors may become malignant.

Immunity.—Information is lacking on human immunity to blood fluke infections, though it is known that infections are most severe in children and uncommon in late adult life. Animals cured of an initial infection show increased resistance to reinfection. Antibodies are demonstrable with extracts of adult worms or heavily infected snail livers. That the reactions are group-specific is shown by the fact that extracts of *Fasciola hepatica* react with sera of infected individuals. The precipitin test is unreliable, but complement-fixation is a dependable tool for diagnosis. A skin test is useful but remains positive long after cure.

Diagnosis.—Laboratory diagnosis preferably is based on the finding of characteristic eggs in urine or feces. Early in infection or after serious tissue damage they may not be detectable, and in such circumstances the immunological tests are of great value.

Antimony compounds, particularly fouadin, are effective in killing the adult worms.

Epidemiology and Control.—Blood fluke infections may be acquired from drinking water, the cercariae penetrating the mucosa of the upper alimentary tract. Occasional infections are also known in newborn infants whose mothers were exposed to infection during gestation. The chief means of infection, however, is by contact of the skin with water containing the cercariae. This occurs especially in the working of rice fields fertilized with human feces, but also in bathing and chance contact. Man is the important reservoir of *Schistosoma mansoni* and *Schistosoma haematobium*, though both are found naturally in monkeys, and other laboratory animals can be infected. *S. japonicum* occurs naturally in a wide variety of animals, including dogs, cats, pigs and cattle. *S. mansoni* is found in Egypt and Central Africa, the West Indies and northern South America, *S. haematobium* in a large part of Africa and in Asia Minor, *S. japonicum* only in the Far East.

Control of blood fluke infections depends on preventing the access of eggs to water containing suitable snails or avoidance of contact with water containing the cercariae. Little success has been attained. Safe sewage disposal or storage of sewage which is to be used for fertilizer is of value. Local destruction of snails is possible with lime or copper salts. Periodic drying of irrigation ditches is also effective except in the Far East, where the snail hosts can withstand long periods of drying.

Other Species.—The lower animal parasites which produce "swimmer's itch" have been mentioned above (p. 738). One of them, *Schistosomatium douthitti*, is of especial interest because it is easily studied in laboratory mice. Several species produce important diseases of domestic animals in Africa and India.

CESTOIDEA

The cestoidea, or tapeworms, generally consist of a colony-like chain of flattened segments, each of which is a semi-independent unit with a complete set of reproductive organs. A *scolex* at the anterior end of the chain serves to anchor the worm in place, and the nervous and excretory systems are shared by the whole organism. There is no alimentary tract, soluble food substances being absorbed through the body surface. All are parasitic, the adults usually in the alimentary tract of the definitive host and the larval stages in the tissues of an intermediate host.

Taenia Solium.—The pork tapeworm, *Taenia solium*, was known in ancient times. Küchenmeister[63] first suspected its relationship to "bladderworms" in pork and demonstrated their transformation into the adult worms in the intestine of a condemned criminal.

Characteristics and Life-Cycle.—In the intestine of man the adult *Taenia solium* attains a length of 2 to 10 meters. Buried in the intestinal mucosa of the host is the scolex, or "head," a rounded cubical organ about 1 mm. in diameter bearing four large, cup-like suckers and a pad, the rostellum, with twenty to thirty-five hooks (Fig. 207). Behind the scolex is a thin neck which merges into a region of proliferation of segments, or *proglot-*

[63] Küchenmeister: Wien. Med. Wchnschr., 1855, *5*:1.

tides. As new segments are produced, the older ones are pushed back, developing sexually, until near the middle of the worm mature proglottides are found. They average 0.5 cm. square. The genital pore occurs on either side of the proglottis, alternating irregularly from segment to segment. The male reproductive system consists of small follicular testes scattered throughout the dorsal part of the segment, emptying through vasa efferentia into a coiled vas deferens which ends in the muscular copulatory organ, or cirrus, at the genital pore. In the female system the vagina extends from the genital pore to the oötype in the posterior central part of the segment. On either side of the oötype are ovaries and behind it the vitellaria. A blind uterus extends forward from the oötype.

As the segments move toward the posterior end of the worm, the uterus fills with eggs, becoming branched to fill most of the segment in the *gravid* proglottis, which is about 0.5 x 1 cm. in size. In *T. solium* there are about

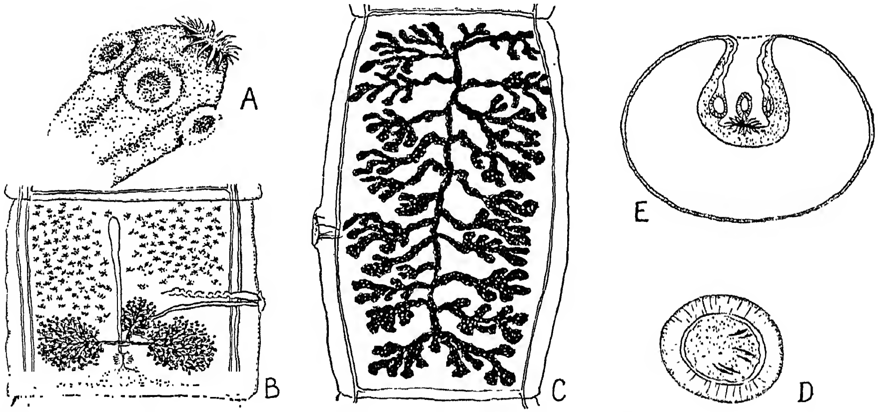


Fig. 207.—*Taenia solium. A*, Scolex; × 20. *B*, Mature proglottis; × 5. *C*, Gravid proglottis; × 5. *D*, Egg; × 500. *E*, Cysticercus; × 5 (Adapted from various sources).

ten branches on each side of the uterus. The gravid segments become detached singly or in small groups and pass out with the feces. Before or after escape from the body they burst, releasing large numbers of eggs. These eggs, averaging 35 μ in diameter, are nearly spherical and provided with a thick shell of characteristic porous structure. An embryo bearing six hooks may be seen within the shell.

Ingested by a suitable intermediate host, the eggs hatch in the intestine and the embryos penetrate the intestinal wall to enter the blood or lymph. They are carried to various parts of the body, developing in the tissues in about two months to the infective stage. Infection is most intense in the muscles, but other tissues also contain the parasites. This stage, a *cysticercus*, consists of a scolex like that of the adult, a short neck, and a fluid-filled sac about 1 cm. in diameter. Because of the sac, this larval stage is commonly called a "bladder-worm." In the tissues the scolex is invaginated into the bladder. In addition to the pig, other animals may harbor the

cysticerci. Among them is man, in whom they occur in various tissues, including the brain.

Man acquires the adult tapeworm by ingestion of undercooked pork containing the cysticerci. In the intestine the bladder is digested away, the scolex attaches to the intestinal wall, and the adult worm develops by growth from the neck region. By two months after infection gravid proglottides containing eggs are being passed in the feces. Infection may last for many years.

The Human Disease.—Adult pork tapeworms usually occur singly in the intestine of man. They often cause no noticeable symptoms, though general intestinal disturbances may occur and in children nervous disturbances are sometimes seen. The ravenous appetite popularly associated with tapeworm infections is actually uncommon, loss of appetite being more frequently observed. In contrast with infection by the adult worm, tissue infection with the cysticerci is dangerous. The larvae act like benign tumors; growth is slow and terminates naturally with full development. The injury results from pressure and its seriousness, therefore, depends on the location of the larvae. In most muscles and connective tissues they are of no consequence, but larvae in the eye may affect vision. In the brain they may give rise to epilepsy or other manifestations of local pressure.

There is no evidence of acquired *immunity* to *T. solium* in man, though it has been suggested that the rarity of multiple infections in the intestine indicates immunity to superinfection. In individuals harboring the cysticercus, complement-fixing and precipitating antibodies are detectable. They react with extracts of other tapeworms as well as *T. solium.*

Laboratory *diagnosis* of intestinal infection with *T. solium* is based on recovery of eggs or gravid proglottides from the feces. The eggs are not distinguishable from those of the beef tapeworm, but the gravid segments can be identified by the smaller number of uterine branches, about ten on each side as against more than fifteen in the beef tapeworm. Diagnosis of the cysticercus infection is rarely made before operation or necropsy, but the immunological reactions mentioned above have been used. Extract of male fern is effective in expelling the adult worm. There is no treatment known for the larval infection except surgery.

Epidemiology and Control.—The larvae of *T. solium* occur most commonly in pigs but are found also in man, monkeys, sheep, camels and dogs. Before 1850 in Berlin 2 per cent of human autopsies showed the cysticerci, but the incidence is now much lower. Pigs acquire the infection by contamination of their food or water with human feces. Larval infections in man are incurred chiefly by contamination of food, water or fingers with eggs from human feces, but it is also possible that in the intestine of an individual harboring the adult worm the eggs may hatch without reaching the outside. Whether or not eggs must leave the body before they are infective, a person with the adult worm in his intestine is a constant hazard to himself as well as to others. Man, the only known host for the adult tapeworm, acquires the infection by ingestion of undercooked pork, which often contains large numbers of larvae. Freezing and thorough cooking kill the larvae, but ordinary pickling and smoking are ineffective. Govern-

ment meat inspection reveals most infected carcasses and has been the most effective single control measure. Other control methods are directed at the prevention of contact between pigs and human feces.

Taenia Saginata.—The beef tapeworm, *Taenia saginata*, resembles *T. solium* in morphology and life cycle, but several differences are noteworthy. The scolex is "unarmed," lacking the hooked rostellum of *T. solium*. The adult worm is usually about 5 meters long, but occasionally attains a length of 25 to 30 meters. The gravid proglottides are distinguished from those of *T. solium* by a larger number of uterine branches, usually 15 to 20. Man is the only known host of the adult. The cysticerci develop in cattle and other ruminants, human infection resulting chiefly from consumption of undercooked beef. Rare cases of human infection with the larvae have been claimed, but it is probable that they were aberrant cysticerci of *T. solium*.

Echinococcus Granulosus.—The adult of *Echinococcus granulosus* inhabits the intestines of dogs and related species. It is a minute worm 0.25 to 0.5 cm. in length, consisting of an "armed" scolex and three proglottides, one immature, one mature and one gravid. Large numbers of the adults may occur in the intestine of an infected dog.

The natural intermediate hosts are sheep, cattle and other ruminants, but a wide variety of animals are susceptible, including man, in whom the larva causes a serious disease. This larva is markedly different from those of the beef and pork tapeworms discussed above. In the viscera of an infected animal it attains a diameter of about 1 cm. after five to six months. It is infective after eight months or more but continues to grow, often, after several years, reaching a diameter of more than 20 cm. The larva, known as a "Hydatid cyst," is a spherical, fluid-filled sac composed of a thick cuticular wall with a thin *germinative epithelium* on its inside surface. From this germinative epithelium are formed two types of structures. Buds may appear and grow into stalked vesicles, *brood capsules*, on the inner surface of which stalked cysticercus-like "scolices" are produced. These brood capsules may become detached, enlarge and produce brood capsules and scolices within themselves, when they are known as *daughter cysts*. In the large cysts there may thus be produced many thousand infective scolices, each of which can develop into an adult worm if the cyst is eaten by a dog feeding on the viscera of an infected animal. Thus *E. granulosus* differs from *T. solium* chiefly in the fact that multiplication occurs in the larval as well as the adult stage. The predominant site of larval infection is in the liver. The lung is next in importance, and cysts may occur also in practically every other organ.

Hydatid Disease in Man.—Echinococcosis, or hydatid disease, is characterized by two general types of manifestations. First, hypersensitivity develops to components of the hydatid fluid and accidental rupture of a cyst may cause serious, even fatal, reactions. Second, and most important, the growing cyst acts like a tumor, the injury resulting from pressure effects and depending on the localization of the cyst. Rupture of a cyst may give rise to new cysts produced from scolices, daughter cysts, or fragments of germinative epithelium. Various abnormalities occur of which

the most important are *multilocular* cysts. These are anomalous in structure, consisting of numerous irregular, jelly-filled cavities which grow irregularly and spread throughout the host tissues like a malignant tumor, usually resulting in death of the host. The majority of multilocular cysts and some unilocular cysts are sterile, producing no scolices.

There is no information available concerning acquired *immunity* to the larval infection in man, though in immunized sheep the cysts develop somewhat abnormally. Dogs injected with antigens derived from the larvae are immune to the adult infection.[64] The hypersensitivity mentioned above can be detected by skin tests with extracts of the cyst fluid. This is the most sensitive immunological test, but complement-fixation and precipitation are also demonstrable.[65] These procedures are somewhat less dependable for laboratory diagnosis than microscopic examination of aspirated cyst fluid, but the puncture of a hydatid cyst is exceedingly hazardous to the patient.

Epidemiology and Control.—The normal life-cycle of *Echinococcus granulosus* involves dogs and sheep or cattle, man being an accidental host of the larva. For this reason, the infection is most common in the great grazing regions of the world, particularly Australia and New Zealand, North and South Africa, Iceland and southern South America. The incidence in cattle and sheep occasionally exceeds 50 per cent. The multilocular cysts are largely confined to central Europe and Siberia, and this has led some investigators to suggest that they are larvae of a different species of worm, *E. multilocularis*. This hypothesis has not been substantiated. It is weakened by the fact that most multilocular hydatids are sterile.

Control of the infection in domestic animals depends chiefly on preventing dogs from eating offal. Possibly infected waste material should be buried or burned. Prophylaxis of human infection requires measures to reduce the chance of contamination of human food and water by feces of dogs.

Other Tapeworms Related to Taenia Solium.—Species of the genus *Multiceps* infect dogs as adults and sheep and other lower animals as larvae. The larva, known as a *coenurus*, resembles a cysticercus but has a number of scolices attached to a single bladder. In the brains of sheep the larvae produce a fatal disease, and occasional cases are known in man.

Taenia pisiformis is a common tapeworm of dogs and cats, the larvae developing in the abdominal cavity of rabbits. *Taenia taeniaeformis* occurs commonly in the intestine of cats. The larvae in the liver of rats and mice are often associated with malignant tumors. This species is of interest for a number of immunological phenomena. Definite sex resistance is shown by the fact that female rats acquire fewer larvae from a given dose of eggs than do males. Castration of females or injection of male sex hormones reduces their natural resistance, while female hormones increase the resistance of the males.[66] Age resistance is also demonstrable in rats. Acquired immunity is induced either by previous infection with the larvae

[64] Turner, Dennis and Berberian: Jour. Egyptian Med. Assoc., 1935, *18*:536.
[65] *Cf.* Dennis: Jour. Parasitol., 1937, *23*:62.
[66] Campbell and Melcher: Jour. Infect. Dis., 1940, *66*:184.

or by immunization with worm material.[67] This immunity is dependent in part on antibodies transferable with serum. Two antibodies are detectable. One acting early in the infection prevents development of the larvae; it is elicited by both infection and vaccination and is removed from the serum by absorption with worm substance. The other acts late in the infection, aborting the development of the larvae in the liver, is detectable only after infection and is not absorbable with parasite material.[68]

Hymenolepis Nana.—The human dwarf tapeworm, *Hymenolepis nana*, is a small worm 1 to 4 cm. in length with the mature proglottides 0.5 to 1 mm. in breadth. The scolex is basically like that of *T. solium* but smaller, about 0.25 mm. in diameter. The mature proglottides are markedly different in appearance from those of *T. solium*, the width being more than four times the length. Three large testes occur in each segment. The uterus in the gravid segments is a large, irregular sac. The gravid segments are usually destroyed in the intestine, releasing the eggs.

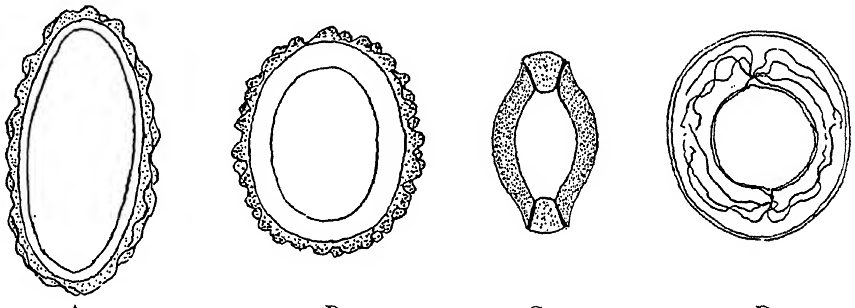


Fig. 208.—Shells of some helminth eggs; × about 400. *A, Ascaris lumbricoides* (infertile). *B, Ascaris lumbricoides*. *C, Trichuris trichiura*. *D, Hymenolepis nana*.

H. nana is unique among human tapeworms in not requiring an intermediate host. The eggs (Fig. 208) hatch in the intestine of man to release an embryo which invades the tissues of an intestinal villus. Here, in about four days, it develops into a *cysticercoid*, a larva with a small bladder and a solid tail posteriorly. This larva breaks out of the intestinal wall, attaches, and develops into the adult worm. Eggs are found in the feces about one month after infection. It is reported that several types of insects can serve as intermediate hosts, but they are probably unimportant in human spread of the parasite. Large numbers of worms are commonly found in an infected individual, and intestinal and nervous disturbances occur in children. Male fern extracts eliminate the worms.

Man, monkeys and rodents are naturally infected. The rodent form has been called *H. fraterna* but is generally considered a variety of *H. nana*.[69] The parasite is cosmopolitan. It is much the most common tapeworm of man in the United States, especially prevalent in children, but occurring

[67] Miller: Proc. Soc. Exp. Biol. & Med., 1930, *27*:926; *ibid.*, 1931, *28*:467.
[68] Campbell: Jour. Immunol., 1938, *35*:205.
[69] Shorb: Amer. Jour. Hyg., 1933, *18*:74.

in all age groups. Age resistance has not been shown in man but is demonstrable in rats and mice.

The lack of an intermediate host permits direct spread from man to man. It is not known whether the eggs can hatch without having reached the outside. However, an infected individual is particularly liable to reinfection from fingers contaminated with his own feces. In this respect *H. nana* differs from the great majority of worm infections, which are numerically stable in the absence of reexposure.

The closely related *H. diminuta* of rats and mice is transmitted by several species of insect intermediate hosts. Occasional human infections have been reported, mostly in children.

Tapeworms of Lower Animals.—*Dipylidium caninum*, a common parasite of the intestines of dogs and cats, measures 10 to 50 cm. in length. Each elongate, rounded mature proglottis contains two sets of reproductive systems, one opening on each side of the segment. The uterus breaks up

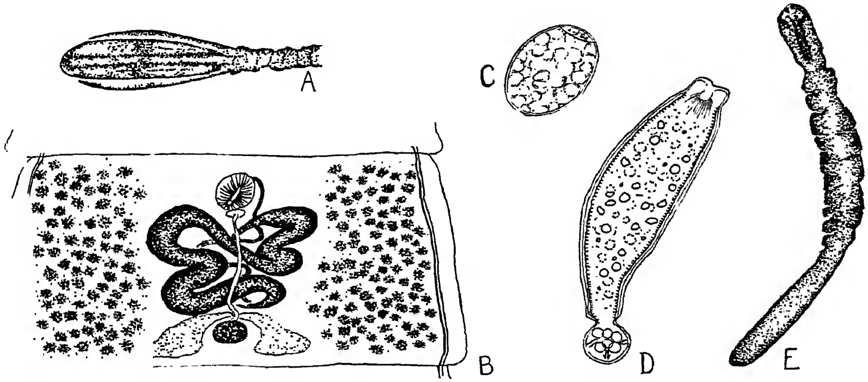


Fig. 209.—*Diphyllobothrium latum*. *A*. Scolex; × 10. *B*, Mature proglottis; × 6. *C*, Egg; × 250. *D*, Procercoid; × 100. *E*, Sparganum: × 8. (Adapted from various sources.)

into pouches, each containing several eggs. Dog lice and various species of fleas serve as intermediate hosts, the cysticercoids in the insects being infective when ingested. Young children occasionally harbor the adult tapeworm.

Rare human infections with a number of other tapeworms of lower animals have been reported.[70]

Diphyllobothrium Latum.—The fish tapeworm of man, *Diphyllobothrium latum*, shows radical differences in structure and life-cycle from the species discussed above (Fig. 209). It is a more primitive type, in many respects showing strong resemblances to the trematodes.

Morphology and Life Cycle.—The adult worm in the intestine of man is very large, often exceeding 10 meters in length. The scolex is an elongate ovoid structure, about 1 by 2.5 mm. in dimensions, bearing, instead of the

[70] *Cf.* Faust: *Human Helminthology.* Lea & Febiger. Philadelphia. 1939.

circular suckers of *T. solium*, two elongated grooves which serve as attachment organs. The mature proglottis is broader than long, about 4 by 10 mm. in size. The small testes are scattered throughout the lateral dorsal regions, emptying by fine ducts into the vas deferens. This coiled tube runs from the posterior center of the segment forward to the muscular cirrus, which opens into the ventral genital pore in the center of the anterior region. The vagina extends from the genital pore back to the large oötype in the center of the posterior region. The ovaries lie on either side of the oötype, and the vitellaria are small follicles scattered throughout the lateral ventral regions. Arising from the oötype, the egg-filled uterus coils throughout the center of the proglottis, ending at the uterine pore just behind the genital pore. This permits escape of the eggs without rupture of the proglottis, so that, contrary to the usual situation as seen in *T. solium*, the eggs are released into the intestinal contents and escape in the feces. The spent proglottides at the end of the worm break off and are passed in the feces.

The egg is undeveloped when it escapes in the feces, embryonation requiring a week or more in water. Like the eggs of most trematodes it is ovoid, with an operculum. Average measurements are 65 by 45 μ. Hatching occurs by opening of the operculum to release a free-swimming, spherical, six-hooked embryo covered with long cilia. This embryo, the *coracidium*, dies within a few days unless it is ingested by a suitable small crustacean, one of several species of copepods of the genera *Cyclops* and *Diaptomus*. Losing its cilia, the embryo reaches the body-cavity of the copepod and becomes, in two to three weeks, an elongated solid body with a round tail bearing the six hooks seen in the embryo. This stage, the *procercoid*, is about 0.5 mm. long.

If the infected copepod is eaten by a fish, the procercoid penetrates the wall of the intestine and reaches the viscera or muscles. Here, in a week or more, it transforms into the *sparganum*, a worm-like stage 0.5 to 2 cm. long with a rudimentary scolex at the anterior end. This stage, ingested by man in undercooked fish, develops to the adult worm in the intestine, and eggs may be found in the feces after three weeks or more. If the infected fish is eaten by another fish, the spargana become established in the tissues of the second fish and remain infective for man. Human infections have been known to persist for years.

The Human Disease.—*D. latum* commonly produces multiple infections. The adult worms usually cause no symptoms, though in heavy infections intestinal disturbances may occur, sometimes with generalized edema. A small proportion of infected individuals show severe anemia, indistinguishable from pernicious anemia. It is probable that the infection merely precipitates the condition in susceptible persons. Satisfactory data on acquired immunity are not available. Laboratory diagnosis depends on identification of the eggs in fecal smears, and extract of male fern eliminates the adult worms.

Epidemiology and Control.—The adult worm parasitizes man, cats, dogs, bears and other fish-eating mammals, but man is probably the principal source of infection in endemic areas. Susceptible copepods are widespread,

and a variety of fish can serve as second intermediate hosts. Endemic centers are known in northern and central Europe, northern Asia, Japan, parts of Africa and the Great Lakes region of North America, many local populations showing 100 per cent infection. Personal protection depends on thorough cooking or freezing of fish from endemic areas and care in handling of such fish. Other control methods are aimed at the avoidance of water pollution with untreated human feces and at the supervision of transportation of possibly infected fish.

Related Species.—Several related species of tapeworms, normally parasites of lower animals, are reported occasionally in man. Man also occasionally harbors the spargana of several species of *Diphyllobothrium.* Infection with these larvae in the subcutaneous tissues, muscles or eye is known as sparganosis. Among the larvae infecting man is *Sparganum proliferum,* which multiplies enormously in the subcutaneous tissues and may cause death. The adult worm of this species is unknown. Larval infections with *Diphyllobothrium* result either from ingestion of copepods in drinking water or from an Oriental practice of using split frogs as poultices. In the latter case, spargana in exposed tissues of the frog may enter the tissues of man.

NEMATODA

All the important human parasites in the phylum *Nemathelminthes* belong to the class *Nematoda.* They are cylindrical, elongated worms, unsegmented and with a false body cavity which is not lined with peritoneum as in higher animals. Symmetry is primitively bilateral. Male and female reproductive systems usually occur in separate individuals noticeably different in general appearance. Many are free-living and the parasitic species occur in a great variety of plants and animals.

A number of nematodes and other Nemathelminthes of lower animals occasionally parasitize man. None is of sufficient importance for inclusion here.[70] The structures and life-cycles of the human parasites are so enormously varied that it will be necessary to concentrate on a few examples. However, the pinworm, *Enterobius vermicularis,* will serve to illustrate the fundamental morphology of the group. A word of caution is necessary here. The order of presentation of species in this section, based primarily on life cycles, cuts sharply across the lines of zoological classification, separating closely related types and bringing together types of very different structure.[71]

Enterobius Vermicularis.—The human pinworm, *Enterobius vermicularis,* normally inhabits the upper large intestine of man, occasionally invading the female genito-urinary system. The female worm, about 1 cm. long by 0.5 mm. in diameter, is a spindle-shaped organism with a long, pointed tail (Fig. 210). The outer covering is a smooth, impervious, flexible cuticle, characteristic of nematodes in general. This cuticle, together with the fact that the body musculature is exclusively longitudinal, gives the worms a characteristic bending, wriggling type of movement. Among nematodes the cuticle shows great variety of external structures—knobs, fins,

[71] For a detailed account of the nematodes *cf.* Chitwood and Chitwood: *An Introduction to Nematology.* Monumental Printing Co. Baltimore. 1937.

papillae, etc.—which are of value in technical identification of species. The alimentary tract consists of a mouth at the anterior end, a muscular esophagus with a posterior bulbous enlargement, and a thin-walled intestine emptying through the anus near the posterior end.[72] The female reproductive system empties through a short vagina a little in front of the middle of the body. Extending forward and back from the vagina are two separate egg-producing systems. Each consists of a broad uterus filled with eggs, followed by a short narrow oviduct, which terminates in a long, thin ovary coiled about in the body cavity.[73] The male is smaller, 2 to 5 mm. in length and 0.1 to 0.2 mm. in diameter, the posterior end coiled into a tight spiral and lacking the pinlike tail of the female. The male reproductive system is a single tube consisting of a testis, a vas deferens, a seminal vesicle and a muscular ejaculatory duct, provided with glands, which empties with the intestine into a cloaca. A sharp copulatory spicule can be extruded through the anus. The posterior end has minute expansions (more prominent in many other nematodes) to form a clasping *bursa* used in copulation.

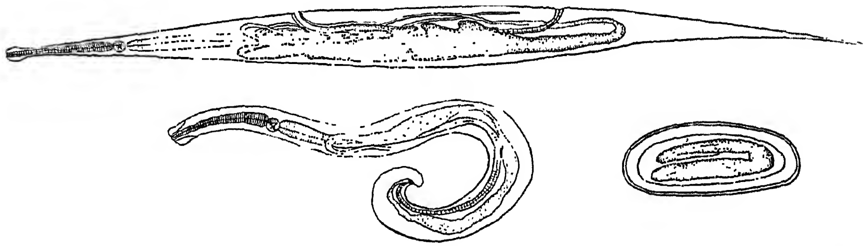


Fig. 210.—*Enterobius vermicularis.* Above, female, × 15. Left, adult male, × 15. Right, egg, × 300. (Redrawn from Faust after Leuckart).

Some of the eggs escape in the intestine, passing out in the feces, but usually the gravid females migrate out of the anus and disintegrate on the surrounding skin to release their eggs. The eggs are infective immediately. Ingested by man they hatch in the small intestine, where the worms mature and mate before passing down to the large intestine. Eggs appear in the feces about two weeks after infection. The adults in the intestine are short-lived but infection is maintained or increased in the individual by self-infection and perhaps by hatching of eggs before escape from the intestine.

Pinworm Infection in Man.—The adult worms in the intestine have little or no effect on the host. However, they occur in the vermiform appendix, and since they are said to be more common in diseased than in normal appendices, they have been suspected of an etiologic rôle in appen-

[72] Some nematodes, such as *Trichinella spiralis*, have a long non-muscular esophagus consisting of a thin-walled, narrow tube embedded in a row of characteristic large cells.

[73] This type of egg-producing system is the rule in nematodes, but the number of such systems emptying into the vagina is variable from one to several, the human parasites showing one or two.

dicitis.[74] The major symptoms result from disintegration of the females on the perianal skin. The consequent intense itching may induce insomnia and nervousness. Bacterial infection of the inflamed skin may result from scratching. There is no evidence of acquired immunity.

Diagnosis is based on identification of eggs in the feces or, more dependably, in scrapings from the perianal skin. For the latter, the NIH swab, a square of cellophane examined microscopically after being rubbed over the skin, is particularly useful. Several drugs, particularly gentian violet and hexylresorcinol, are effective in eliminating the worms.

Epidemiology and Control.—Man is the only host of *E. vermicularis*. The eggs are resistant to drying and may widely contaminate clothing, bedding and house dust, thus maintaining a constant source of infection in homes and institutions. The parasite is cosmopolitan. It is especially common in children, probably largely because their unsanitary habits allow greater chances of spread. Treatment of cases is useful in control, provided sanitary measures are taken to minimize reinfection from the local environment.

Related Species.—Similar nematodes occur in many lower animals and are sometimes economically important as minor disease agents. Species in rodents are often easily obtained for study as are several related parasites of cockroaches.

Trichuris Trichiura.—*Trichuris trichiura* is a frequent inhabitant of the cecum, appendix and upper colon of man. The anterior three-fifths of the adult worm, containing the non-muscular esophagus, is attenuated, and the general appearance of the body has given rise to the common name "whipworm." Male and female worms are similar in size, 3 to 5 cm. long.[75] The adult worms, embedded in the intestinal mucosa, have been accused of a rôle in the pathogenesis of appendicitis, but the evidence is inconclusive. In heavy infections they may cause intestinal disturbances and general toxic effects. Treatment with wild fig latex eliminates the worms. The eggs passed in the feces are thick-walled with a plug at either end, 53 by 22 μ in size (Fig. 208). They are very resistant, surviving for months in contaminated soil. The embryos, undeveloped at the time of passage, require two weeks to several months, depending on temperature and humidity, to reach the infective stage. Ingested with contaminated food or water, they produce adult worms in the intestine. *Trichuris trichiura* infection is worldwide but especially abundant in the tropics and subtropics because of poor sanitation and the effect of climate on development of the eggs.

Ascaris Lumbricoides.—Widely known simply as the "roundworm" of man, *Ascaris lumbricoides* is a large parasite of the small intestine. The white or flesh-colored females measure 20 cm. or more in length by 5 mm. in diameter, the males, 16 cm. by 3 mm. The characteristic eggs, 45 to 75 by 35 to 50 μ, have a smooth inner and a roughly tuberculated outer shell and are undeveloped when passed in the feces (Fig. 208). In soil or water they become infective in a week or more, depending on environmental conditions, but may survive for several years despite drying, bac-

[74] *Cf.* Rector: Amer. Jour. Trop. Med., 1943, *23*:369.

[75] The basic morphology of *T. trichiura* is similar to that of *Trichinella spiralis*, page 757.

terial contamination or adverse chemical conditions. Ingested in food or water they hatch in the intestine. The embryos, however, do not develop directly to adults in this site. Gaining access to the circulation they are carried to the lungs and escape into the air spaces. Via the trachea they reach the pharynx and are swallowed. Partial development has occurred during the above migration. It is completed in the small intestine, where egg-producing adults are found about two and one-half months after infection.

Passage of the migrating larvae through the lung may produce a severe broncho-pneumonia. Larvae filtered out of the circulation in abnormal sites induce local inflammatory lesions which are serious in certain locations, such as the brain. Adult worms in the intestine often cause no symptoms. Particularly in heavy infections, however, intestinal and nervous disturbances may occur, and intestinal obstruction occasionally results. Various other injuries result from migration of the adult worms out of the normal habitat into the appendix, the bile ducts, the upper alimentary or respiratory tracts, the genito-urinary system or through the intestinal wall into the abdominal cavity. Hypersensitivity develops in both infected persons and biologists who have handled the worms, and severe anaphylactic reactions may occur in such individuals.

Immunity to reinfection is not known in man but infection with related parasites in animals confers some temporary protection. Antibodies are detectable by various tests after either infection or vaccination with worm extracts, and considerable investigation has been devoted to antigenic analysis. An allergic skin test has been used in diagnosis but is often positive in uninfected individuals who have acquired a hypersensitivity from contact with the parasite. Laboratory diagnosis is preferably based on the finding of eggs in stools. However, a small proportion of cases harbors only male worms, and in such cases the immunological tests can be used.

Oil of chenopodium and hexylresorcinol are effective in removing the adult worms from the intestine. Some other drugs, particularly those used against hookworms (p. 753), cause the dangerous migration of adult worms mentioned above.

Man is the only known host of *A. lumbricoides*. Infection predominates in children; that this results in part from age resistance is suggested by the marked age resistance of animals to related parasites. Infection is world-wide but most prevalent in the tropics and subtropics, where sanitary and climatic conditions favor its dissemination. Control depends on the reduction of soil contamination by proper sewage disposal and treatment of infected individuals. Raw vegetables are an important source of the eggs and should be thoroughly washed.

Related Species.—Cats, dogs, cattle, horses and other lower animals harbor a number of closely related species. Rare human infections have been reported with at least three of these. A distinct race of *A. lumbricoides* causes a severe pneumonitis in young pigs, known as "thumps." In man the eggs hatch and the larvae reach the lung but adult worms do not become established in the intestine. Similarly, the human *A. lumbricoides* develops

only to the lung stage in pigs.[76] The adult worms are easily obtained from slaughter houses for laboratory study.

Hookworms.[77]—The two important hookworms of man, *Ancylostoma duodenale* and *Necator americanus*, will be discussed together. Known since antiquity, human hookworms were first accurately described by Dubini[78] in 1843. Their relationship to disease was definitely shown by Perroncito[79] in 1880, and the life-cycle was elaborated by Looss.[80]

Characteristics and Life-Cycle.—The adults of *A. duodenale* from the human small intestine are shown in Fig. 211. The female measures 1 to 1.5 cm. in length by about 0.5 mm. in diameter. The double reproductive system coils abundantly throughout the body cavity. Two pairs of promi-

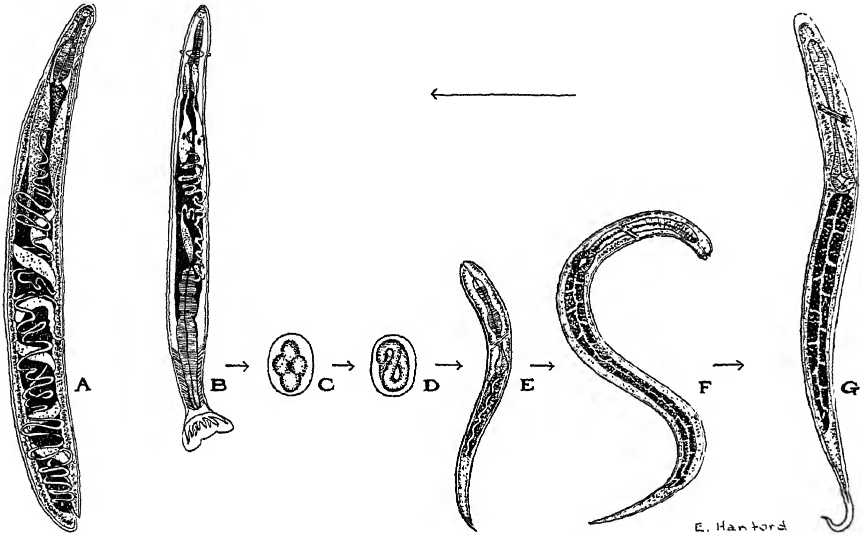


Fig. 211.—Life-cycle of a hookworm, *Ancylostoma duodenale. A,* Female worm. *B,* Male worm. *C* and *D,* Eggs. *E,* First stage larva. *F,* Second stage larva. *G,* Infective larva. *A* and *B*; × 10: *C–G*; × 250 (Adapted from Looss).

nent unicellular glands are seen in the anterior half of the body, one excretory in function, the other secreting histolytic enzymes. The dorsally directed mouth is characterized by two pairs of ventral teeth, whereas in *N. americanus* these teeth are replaced by curved cutting plates. The male measures a little less than 1 cm. in length by about 0.4 mm. in diameter. The reproductive system shows a long, muscular ejaculatory duct, a prominent seminal vesicle and a coiled testis. At the posterior end is a broad,

[76] Koino: Japan Med. World, 1922, *2*:317.
[77] For a comprehensive book see Chandler, *Hookworm Disease*. The Macmillan Company. New York. 1929.
[78] Dubini: Ann. Univ. di Med., Milano, 1843, *106*:5.
[79] Perroncito: Atti. Acad. d. Lincei, Roma, 1880, *7*:381.
[80] Looss: Ministry of Education, Egypt, Records of the School of Med., *3, 4*.

flat clasping apparatus, the copulatory bursa, composed of cuticle supported by finger-like rays of tissue. *A. duodenale* and *N. americanus* can be identified by details of structure of this bursa as well as by the differences in buccal teeth noted above.

The thin-walled eggs measure about 38 by 58 μ and are indistinguishable in the two species. They are usually in early stages of development upon escape from the body, maturing and hatching within twenty-four hours if conditions are favorable in the soil. The larva, about 0.25 mm. in length, feeds on fecal material and grows in three days or more to a length of about 0.4 mm. After moulting it continues to grow until, by the fifth day or later, it measures about 0.6 mm. These two stages, characterized by a bulbous esophagus, are known as *rhabditiform* larvae. The third stage, resulting from a second moult, is a thinner form with a closed mouth and a finer esophagus. It is usually still surrounded by a "sheath," the moulted skin of the preceding stage. This is the *filariform* larva, the infective stage for man. It does not feed but may survive long periods in the soil under favorable conditions. If the infective larva comes in contact with human skin, it actively penetrates through abrasions or hair follicles, reaches the circulation and is carried to the lungs. Here, like the migrating larva of *Ascaris*, it breaks out into the air spaces and reaches the intestine via the trachea and esophagus. After two further moults, the worms are mature. Egg production begins about one month after infection.

Hookworm Disease.—Penetration of the skin by hookworm larvae produces a dermatitis known as "ground itch" or "miner's itch." The adult hookworms live attached to the mucosa of the small intestine, feeding on blood and bits of tissue. They move about frequently, leaving bleeding wounds in the intestinal mucosa. The chronic loss of blood causes anemia and edema, resulting, in severe cases, in retardation of growth and mental development and in weakness and general debilitation. Intestinal disturbances are seen in heavy infections. Death is rarely a direct result of hookworm disease, but the condition often contributes to death from other causes. In white persons the symptoms are directly related to the intensity of infection, which, because of the necessary development outside the body, is stable unless reexposure occurs. Individuals with fewer than twenty-five *N. americanus* virtually never show symptoms. Those with between twenty-five and one hundred worms show borderline effects. With more than one hundred worms some general injury is almost always detectable. *A. duodenale* is more harmful, about half as many worms being required to produce a given effect. Normally, infection is maintained by constant reexposure. In unexposed prisoners, however, it has been shown that about one half of the worms are lost in six months and about three quarters in two years. A smaller number persist for more than five years.

Immunity.—Puppies can be largely protected against the dog hookworm by repeated small infections.[81] Recently similar partial protection has been reported in a human volunteer.[81a] That antibodies are involved is shown

[81] Otto and Kerr: Amer. Jour. Hyg., 1939, Sec. D *29*:25; Otto, J. Parasitol. (Suppl.), 1938, *24*:10.

[81a] Otto: Amer. Soc. Trop. Med. meeting, St. Louis, November, 1944.

by the fact that some protection is conferred on puppies by immune serum.

DIAGNOSIS.—Diagnosis of hookworm infection is based on recovery of characteristic eggs from the feces. Direct fecal smears were formerly used, but many infections with fewer than twenty-five worms are not detected with this method. Essentially all infections are revealed by the Willis flotation method, in which the eggs rise to the surface of a brine suspension of feces and are collected on a large microscope slide inverted over the container. Because the number of hookworms present in an infected individual is so important, several quantitative diagnostic methods have been devised. In the Stoll method a measured quantity (4 cc.) of feces is mixed with dilute NaOH solution (56 cc.) and all the eggs in a known sample volume (0.075 cc.) of this suspension are counted. The Lane method, or D.C.F. (direct centrifugal flotation) can be applied to lighter infections. One cubic centimeter of feces washed by centrifugation is mixed with brine and recentrifuged with a cover slip on top of the tube. The eggs rise and can be counted on the cover slip as in the Willis method. Figures obtained by either of these methods are expressed as the number of eggs per gram of feces. A single female *N. americanus* produces about forty-five eggs per gram of feces (about 6000 eggs per day) so that twenty-five worms (males and females) produce about 600 eggs per gram and 100 worms produce about 2500 eggs per gram. *A. duodenale* produces about twice as many eggs per worm, but because of the greater pathogenicity of this species the egg output gives a roughly equivalent index of the severity of infection in both species.

A number of drugs are effective in eliminating hookworms from the intestine. The best at present are tetrachlorethylene and carbon tetrachloride.

Epidemiology and Control.—Worms morphologically similar to *N. americanus* occur in pigs, but infection experiments indicate that they are distinct from the human hookworms. Man is the reservoir of infection with both *A. duodenale* and *N. americanus.*

There has been much study of the habits and requirements of hookworm larvae in the soil. The optimal temperatures are about 75° F. for *A. duodenale* and about 80° F. for *N. americanus.* Consistent high temperatures, above 100° F., are unfavorable. At 60° F. development is prolonged to two weeks or more, and below 50° F. there is little or no development. Continued temperatures below 40° F. kill the larvae. Moisture is essential to survival of the soil stages, but they die under water and are scattered by heavy rain. The type of soil is significant, probably largely in relation to its waterholding properties. Coarse sand and heavy clay are unfavorable, optimal development occurring in light sand or sandy loam. A large proportion of the infective larvae die within the first two weeks but a few survive for several months under favorable conditions. Although lateral migration from the site of development is insignificant, the filariform larvae can move considerable distances vertically in the soil. Unless excessive drying occurs, they remain at the surface, where the chance of contact with human skin is greatest.

In addition to the environmental requirements of the larvae, various

human factors affect the distribution of hookworm infection. Fecal disposal practices and the use of shoes are among the most important. All these factors combine to make hookworm infection a problem of the rural parts of warm countries. Exceptions occur in mines and tunnel-building operations, where local conditions may be favorable despite a generally unsuitable environment. *N. americanus* is the hookworm of central and southern Africa, the Western Hemisphere and southern India. Throughout the rest of southern Asia it is mixed with *A. duodenale*, and a few foci of *A. duodenale* occur in South America. In southern Europe, European mines and North Africa only *A. duodenale* is found.

In the southern United States infection is most common in children, largely because adults are protected from infection by wearing shoes. That age resistance, if it exists at all, is a minor factor is shown by the fact that in countries where the whole population goes without shoes infection may predominate in adults. Racial immunity is marked, and Negroes under comparable conditions have fewer and less intense infections than do whites. Furthermore, Negroes seldom show clinical symptoms even in the presence of heavy infections.

Control depends on the reduction of soil contamination by treatment of cases and proper fecal disposal and on the prevention of contact between human skin and the soil. Chemical destruction of larvae in the soil has been of use only in particular cases, such as mines. Education is the major weapon in hookworm control, for the chief problems are sociological, and biological knowledge of the parasite is adequate to insure successful hookworm eradication if the habits of infected populations can be sufficiently influenced. Treatment of cases has an important place in hookworm campaigns. Formerly it was customary to treat all infected members of a community, or even the whole community (mass treatment), if sample diagnoses showed abundant and heavy infections. Current opinion favors treatment of only the clinically significant cases. This has two great advantages. It simplifies the problem of preliminary surveys, since the search for infections may be limited to those population groups most likely to be heavily infected. In the second place, it avoids the educational disadvantages of treating persons who are not visibly benefited by the drug. In most regions the treatment of a small proportion of the population can eliminate most of the egg production.[82]

Hookworms of Lower Animals.—Various animals harbor hookworms. Two species, *Ancylostoma braziliense* and *A. caninum* of dogs and cats occasionally invade the skin of man. They are unable to develop further, and their wandering in the subcutaneous tissues produces a serpiginous dermatitis known as "creeping eruption." The adults of *A. braziliense* have been reported from man in the Far East. Other, more distantly related, parasites are important disease agents of livestock.

Nippostrongylus muris of rats and mice is of interest because of extensive studies on the mechanisms of immunity. Serum antibodies have been shown to exert a direct action on the parasites *in vitro*, and these antibodies to-

[82] Andrews: Amer. Jour. Pub. Health, 1942, *32*:282.

gether with tissue cells immobilize and destroy the larvae in the skin and lungs and destroy adults in the intestine.[83]

Strongyloides Stercoralis.—*Strongyloides stercoralis* is a common intestinal parasite of man in warm countries. It exhibits a life-cycle of extreme complexity, involving reproduction by adults in the soil as well as in the human intestine. Investigators working with different animal species of *Strongyloides* disagree on fundamental points and it is not clear whether or not these disagreements result from specific differences in the worms studied.

Only females are known in the intestine of man. They are thin, transparent worms 2 to 2.5 mm. long by about 0.05 mm. in diameter. They lie buried in the mucosa of the small intestine and the thin-shelled eggs are deposited in the tissues, where they hatch to release rhabditiform larvae very similar to those of hookworms. Escaping in the feces the larvae undergo one of two types of development. They may moult to filariform larvae which penetrate the skin to establish infection. Alternatively, they may develop into the free-living males and females. The free-living female is stouter than the parasitic female and about 1 mm. long. The male is somewhat smaller, about 0.7 mm. in length. Eggs produced by the free-living female hatch to release rhabditiform larvae which may continue the free-living cycle or may develop into infective larvae. The infective larvae, whether produced by free-living adults or developed directly from the rhabditiform larvae in the feces, penetrate the skin of man and reach the intestine via the circulation and lungs as in the hookworms. Male worms have not been found in the intestine, but "parasitic" males like those in the soil have been reported[84] in the lung, and it has been suggested that fertilization occurs here or en route to the intestine, the males being quickly lost.[85] Since investigation of a related species in rats has shown that a single infective larva can give rise to a female in the intestine producing fertile eggs, it is clear that the parasitic males are not essential in the life cycle.[86] Oviposition by females in the lung has also been reported.[87] A final complication is introduced by the reports that rhabditiform larvae might transform into infective larvae in the colon and penetrate its wall or the perianal skin to produce hyperinfections.[88] The various paths of development indicated above are shown in Fig. 212, the dotted lines indicating phenomena which are incompletely known.

Penetration of the skin by the larvae causes dermatitis, and passage through the lung may give rise to a bronchopneumonia. The parasitic females cause intestinal disturbances characterized chiefly by diarrhea. Laboratory diagnosis is based on identification of the rhabditiform larvae in the feces. Gentian violet has been reported active against the female worms in the intestine, but there is disagreement concerning its efficiency.

[83] Taliaferro and Sarles: Jour. Infect. Dis., 1938, *64*:35; *ibid.*, 1939, *64*:157.

[84] Kreis: Amer. Jour. Hyg., 1932, *16*:450.

[85] Faust: *Ibid.*, 1933, *18*:114.

[86] Graham: Amer. Jour. Hyg., 1936, *24*:71; 1938, *27*:221; 1939, *30* (sec. D): 15; Jour. Parasit., 1938, *24*:233; 1939, *25*:365.

[87] Fülleborn: Arch. f. Schiffs.-u. Tropen.-Hyg. (Beih.), 1914, *5*:26.

[88] Nishigori: Jour. Form. Med. Soc., 1928, *277*:1.

The epidemiology of *Stronglyloides stercoralis* infection is similar to that of hookworm, and the same sanitary control measures apply.

Trichinella Spiralis.[88a]—The "trichina" worm, *Trichinella spiralis*, was first seen in human muscles at necropsy by Tiedemann in 1821. Leuckart and others worked out the life cycle.[89] The adult worms are intestinal parasites of man and many other mammals, but their sojourn in the intestine is so brief that the more persistent larval infection in the muscles receives the main emphasis. The infective stage for man is a larva in the muscles of the hog. This stage is a minute coiled worm, about 1 mm. in length, enclosed in a lemon-shaped fibrous cyst, 0.25 by 0.5 mm. in dimensions. Ingested by a susceptible host the cysts reach the stomach, where the muscle and cyst wall are digested away. In about two days the larvae mature in

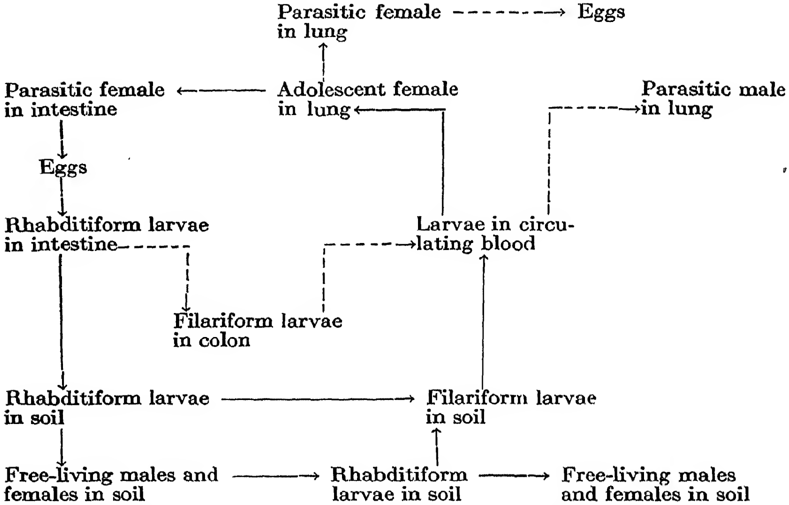


Fig. 212.—Schematic life-cycle of *Strongyloides stercoralis*. Broken arrows indicate rare or incompletely understood possibilities of development.

the small intestine to adult males and females. The male is a thin, transparent worm about 1.5 by 0.04 mm. (Fig. 213). The anterior half of the body is occupied by a nonmuscular esophagus like that of the closely-related *Trichuris trichiura*. At the posterior end are two pear-shaped clasping lobes. The female is about 4 by 0.06 mm. in size. After fertilization the females burrow into the mucosa where, after about one week, they begin to deposit minute larvae, about 0.1 mm. in length, in the tissues. These larvae reach the general circulation and are filtered out in the skeletal muscles. There they grow in about two weeks to the infective stage. One month after infection the larvae are surrounded by a fully developed cyst

[88a] An excellent general discussion is: Gould: *Trichinosis*. Charles C Thomas. Springfield, Ill. 1945.

[89] Leuckart: *Untersuchungen über Trichina spiralis*. Leipzig and Heidelberg. 1866.

wall, produced by the host tissues. Much later, probably only after death of the larvae, these cysts become calcified, but they are known to remain infective at least for several months. Any skeletal muscle may be infected, but the larvae are most abundant in the diaphragm, intercostals, tongue, larynx and eye muscles. Meanwhile the adults have disappeared from the intestine. Most of the males die and are passed out within a week after infection. The females have largely disappeared within a month. It will be seen that the complete life cycle occurs in a single individual, for infective larvae are produced in the tissues of the same host which harbors the adults in its intestine.

Trichinosis.—Trichinosis in man is characterized by two phases. Intense infection with the adult worms produces gastro-intestinal disturbances,

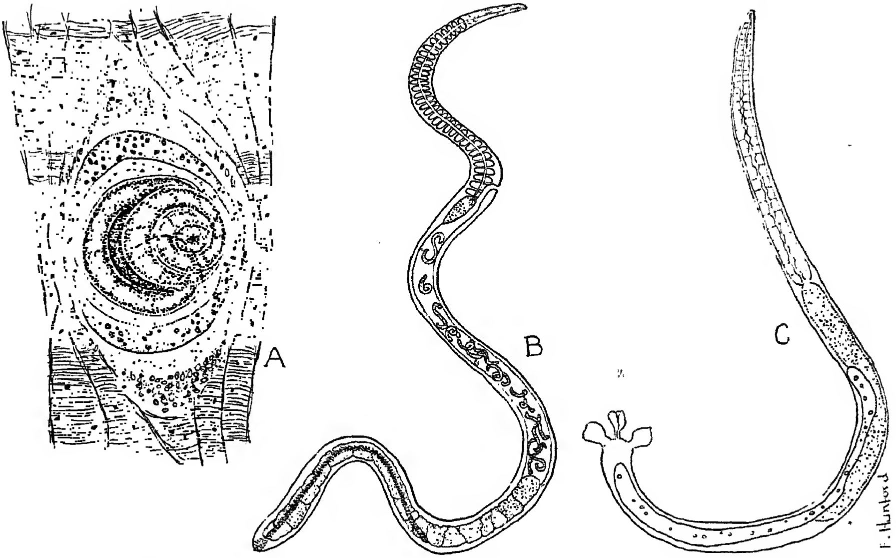


Fig. 213.—*Trichinella spiralis*. *A*, Larva in muscle; × 100. *B*, Adult female; × 50. *C*, Adult male; × 50.

usually with diarrhea. Occasionally the injury to the intestine is so severe as to result in death within a few days after infection. Muscle invasion by the larvae leads to varied signs of toxemia and muscle injury. Heavy infections cause death or chronic illness of long duration. The severity of the disease is proportional to the number of larvae ingested, and many mild cases undoubtedly escape detection.

Immunity to superinfection is demonstrable in experimental animals and can be transferred with immune serum. Some protection may be produced by vaccination with killed larvae but this is insufficient to be of practical value.[90] Direct action of immune serum on the worms has been

[90] McCoy: Amer. Jour. Hyg., 1931, *14*:484; 1935, *21*:200.

described. Precipitin and skin tests are available using extracts of larvae digested out of infected muscle.[91] The precipitin test becomes positive about three weeks after infection, the skin test at about two weeks. They remain positive for several years. Precipitating antigen has been detected in the serum as early as twenty-four hours after infection, and this phenomenon offers possibilities for early diagnosis.[92] Other diagnostic methods involve search for the larvae in body fluids or tissues. During migration they may be found occasionally in blood or spinal fluid, but it is often impossible to detect them. Muscle biopsies show the larvae in severe cases. Unfortunately, even if definite diagnosis is made, there is no known treatment for trichinosis.

Trichinella spiralis occurs principally in man, hogs, rats and bears, although experimental infection has been produced in many species of mammals and birds. Human infection follows the consumption of undercooked pork, while pigs acquire the parasite from garbage containing pork scraps or, less commonly, by eating carcasses of infected rats. *Trichinella spiralis* is world-wide in distribution, though infection in the tropics is rare. In the United States routine necropsy examination of human diaphragms has revealed a general infection rate of between 14 and 28 per cent despite the fact that very few clinical cases are diagnosed.

Personal protection can be assured by thorough cooking of pork products, including hamburger of unknown composition. Government meat inspection does not attempt to detect *Trichinella*, for examination guaranteeing safety is impractical, but refrigeration for twenty-four hours at −18° C. destroys most of the larvae.[93] Laws which forbid the feeding of raw garbage to hogs have been successful in reducing the incidence of infection.

Wuchereria Bancrofti.—Of the several species of "filarial" worms which parasitize man, *Wuchereria bancrofti* is by far the most important. The disease, elephantiasis, was known in antiquity, but the larvae were first seen in 1863 by Demarquay and the adults by Bancroft in 1876. Manson, in 1878, showed that development of the larvae took place in mosquitoes.[94] This discovery is noteworthy because it represents the first incrimination of an insect in the transmission of disease.

Characteristics and Life-Cycle.—The adults live coiled in the lymph nodes of man. They are thread-like translucent worms, the female 7 to 10 cm. long by 0.25 mm. in diameter, the male 4 cm. by 0.1 mm. The egg, covered by a thin membrane, hatches in the uterus or in the host tissues to release an active larva about 0.2 mm. long. This larva, known as a *microfilaria*, escapes into the lymph and enters the circulating blood by way of the thoracic duct (Fig. 214). In most regions the larvae of *W. bancrofti* show marked nocturnal periodicity, occurring in the peripheral blood almost exclusively at night. In many of the South Pacific islands, however, they appear in the blood at all hours. Extensive study has failed to provide a

[91] Bachman: Jour. Prev. Med., 1928, 2:513; 1929, *3*:465.
[92] Bozicevich and Detre: Pub. Health Rep., 1940, *55*:683.
[93] Augustine: Amer. Jour. Hyg., 1933, *17*:697.
[94] *Cf.* Manson: Trans. Linn. Soc. London, 1884, *2*:367.

satisfactory explanation of this periodicity.[95] There is no evidence as to the longevity of microfilariae in infected persons. In normal individuals inoculated with blood containing the microfilariae, they are said to survive for at least two weeks, but the situation may be quite different in persons harboring the adult worms. At any rate, the microfilaria develops no further in the circulating blood. If ingested by a susceptible mosquito, however, it undergoes a series of transformations in the tissues of the insect. Within a day the larva leaves the stomach of the mosquito and invades the thoracic muscles. Here it develops into a stout "sausage larva," which, after a second moult, elongates to form the infective stage, a thin worm 1.5 to 2 mm. in length. This infective larva leaves the muscles and migrates to the proboscis sheath of the mosquito, When the mosquito

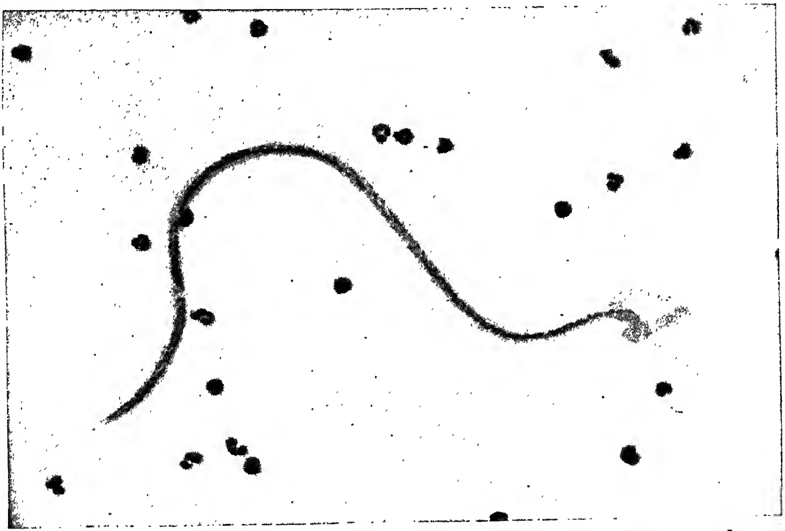


Fig. 214.—Microfilaria of *Wuchereria bancrofti* in thick blood film; × about 150. (Courtesy National Institute of Health, U. S. P. H. S.)

takes a blood meal, the infective larva, apparently stimulated by the warmth of the skin, breaks out of the proboscis sheath and penetrates the skin, probably through the wound caused by the biting mosquito, to establish infection in man. The early development of the worms in man is not known, but microfilariae appear in the circulating blood several months after infection. Although the longevity of the adult worms in the lymphatics has not been determined, they probably live for several years.

Filariasis.—The adult worms are most prevalent in the lymph nodes of the inguinal region, but they are also found in lymphatics elsewhere in the body. They induce inflammation and fibrosis in the infected nodes. The consequent restriction of lymph flow causes edema, lymphangitis,

[95] *Cf.* Hinman: Jour. Trop. Med. and Hyg., 1937, *40*:200.

lymphadenitis and elephantiasis. Secondary infection of the lymphatics with streptococci and staphylococci is often significant. Long-standing cases sometimes show enormous enlargement of the scrotum, vulva or legs.

Evidence of acquired immunity is lacking, but complement-fixing antibodies are detectable in the serum, and skin tests are positive in infected individuals. The reactions are group-specific, occurring with extracts of filarial worms from lower animals. Laboratory diagnosis is preferably based on the identification of microfilariae in fresh blood preparations or stained thick blood films. They are often not detectable in early infections or in long-standing cases with much tissue damage. Other filarial worms infecting man (see below) also produce microfilariae in the circulating blood, but these can be differentiated microscopically from the larvae of *W. bancrofti*. Certain antimony compounds eliminate microfilariae from the blood, but it is not known whether they destroy the adult worms.[96] Furthermore, it is not sure that living worms are more injurious than dead ones.

Epidemiology and Control.—Man is the only known host of the adult worm. A large number of mosquitoes of the genera *Culex*, *Aedes*, *Mansonia* and *Anopheles* support development of the larvae, but the most important are *Aedes variegatus* in the South Pacific islands, *Anopheles gambiae* in West Africa and *Culex quinquefasciatus* (*C. fatigans*) elsewhere. There is an interesting correlation between the habits of the mosquito hosts and the periodicity of the microfilariae discussed above. *Aedes variegatus* bites by day, and it is in the regions where this mosquito transmits *W. bancrofti* that the microfilariae are found in the peripheral blood at all hours. In other regions, where the principal vectors attack man in the evening or at night, the microfilariae show a marked nocturnal periodicity.

Because of a combination of factors, *W. bancrofti* is a parasite of warm countries, occurring widely throughout the tropics and subtropics. Climate favors the production of mosquitoes and the development of larvae of *W. bancrofti* in the insect, and the poorer housing of the tropics permits greater contact between mosquitoes and man. The slow development of human infections, the lack of multiplication of *W. bancrofti* in the mosquito hosts, and other hazards combine to limit dissemination of the parasite. As a consequence the infection seems to die out in an area unless there are many human cases and abundant mosquitoes. The only endemic center in the United States, around Charleston, South Carolina, where the incidence was 20 to 30 per cent before 1920, now shows very few cases. Control is a matter of mosquito reduction and protection from mosquito bites, attention being directed to the habits of the particular vectors in any area.

Other Species of Filarial Worms.—*Wuchereria malayi*, a species very similar to *W. bancrofti*, occurs in southern Asia, often together with *W. bancrofti*. Various species of *Mansonia* and *Anopheles* are the vectors. *Loa loa* inhabits the subcutaneous tissues of man in Africa and is called the "eye worm" because of its habit of crossing the eyeball. Large temporary "calabar swellings" occur in the skin, but the infection is not serious. The microfilariae from the blood develop in deerflies of the genus *Chrysops*.

[96] Brown: Jour. Amer. Med. Assn., 1944, *125*:952.

Acanthocheilonema perstans and *Mansonella ozardi* are harmless parasites of human connective tissues. They are transmitted by midges of the genus *Culicoides*. *Onchocerca volvulus* lives in nodules in the skin of man in Africa and Central America. The microfilariae do not occur in the blood but are found in the skin. In or near the eye they may cause blindness. Black flies of the genus *Simulium* serve as vectors.[97] Many other filarial worms are found in various mammals and birds. The dog heart-worm, *Dirofilaria immitis*, and several species in cotton rats are much used in laboratory investigation.

The Guinea worm, *Dracunculus medinensis*, differs considerably from the other filarial worms infecting man. The small male worms are rarely seen. The females, after several months' development in internal connective tissues, appear in the subcutaneous tissues, usually of the leg. They are large worms, averaging about 1 meter in length, often visible through the skin as they lie in the tissues. The skin of the host ulcerates at the anterior end of the worm, and larvae escape, usually when the leg is submerged in water. These larvae develop in copepods, and human infection follows ingestion of the parasitized copepods in drinking water. The human infection, possibly the "fiery serpent" of the Bible, is found in Asia, Africa and parts of the West Indies and South America. Various lower animals also harbor the parasite.

[97] *Cf.* Strong *et al.:* Amer. Jour. Trop. Med., 1938, *18*, Suppl. to No. 1.

CHAPTER 35

THE RICKETTSIAE[1]

The generic name *Rickettsia* is applied to a group of very small, gram-negative coccobacillary microorganisms which are the etiologic agents of the typhus fevers, spotted fever and related diseases, and tsutsugamushi disease of the Far East. They were first observed by Ricketts[2] in 1909 associated with Rocky Mountain spotted fever. Those found in bodies of lice taken from typhus fever patients by da Rocha-Lima[3] in 1916 were named by him *Rickettsia prowazeki* in honor of Ricketts, who had died in 1910 of typhus fever during an investigation of that disease, and of von Prowazek, another early worker in the field who died of typhus.

Morphology and Staining.—The rickettsiae are small coccobacillary forms and may appear either as cocci or as short bacilli. They are usually 0.3 to 0.5 μ long and 0.3 μ wide, but bacillary forms may be as long as 2 μ. Examination with the electron microscope has indicated that they closely resemble the bacteria in internal structure, being homogeneous or slightly granular.[4] They occur singly, in pairs, and not infrequently in dense irregular masses in which no particular arrangement is apparent. Although sometimes found occurring free in the bodies of insects and infected persons and animals, the pathogenic forms proliferate only intracellularly and are usually observed, sometimes in large dense masses (Fig. 215), within the cells, particularly those of mesothelial origin which line the serous cavities. Some species of rickettsiae are found only in the cytoplasm while others invade the nucleus. They are non-motile and non-encapsulated. They are usually said to be non-filterable but *Rickettsia diaporica* readily passes diatomaceous earth filters.

The rickettsiae stain very poorly or not at all with the usual aniline dyes but readily stain a reddish purple with Giemsa. A buffered methylene-blue stain and safranine counterstain devised by Castaneda[5] stains rickettsiae a light blue. The coccus and coccobacillary forms stain evenly, but bipolar staining of the bacillary forms is not uncommon.

Cultivation.—The rickettsiae resemble the filterable viruses in that, with a single exception, they have not been cultivated in the absence of living cells. The exception is *R. melophagi*, a non-pathogenic form found in the sheep tick *Melophagus ovinus*, which has been grown on glucose

[1] Accounts of recent work on the rickettsial diseases may be found in the chapters by Wolbach, Pinkerton, Gordon and Zinsser in *Virus and Rickettsial Diseases*. Harvard University Press. Cambridge. 1940. See also the review by Pinkerton: Bact. Rev., 1942, *6*:37.

[2] Ricketts: Jour. Amer. Med. Assn., 1909, *52*:379.

[3] da Rocha-Lima: Ber. klin. Wchnschr., 1916, *53*:567.

[4] Plotz, Smadel, Anderson and Chambers: Jour. Exp. Med., 1943, *77*:355.

[5] Castaneda: Jour. Inf. Dis., 1930, *47*:416.

blood agar. Several of the pathogenic forms, however, have been grown in tissue culture, both in the usual plasma-tissue cultures and in various modifications of Maitland's tissue-Tyrode medium. They also develop on the chorioallantoic membrane of the chick embryo. The pathogenic rickettsiae so cultivated remain virulent, and reproduction of the disease with such cultures may be regarded as at least a partial fulfillment of Koch's second and third postulates.

The cultivation of rickettsiae has assumed particular significance as a means of producing large amounts of material for the preparation of vaccines. A number of methods have been devised for securing large numbers of rickettsiae, and, although some of them are not cultivation methods, they may be conveniently considered here. All are recently developed, and it is not as yet possible to assess their practical value for the production of vaccine material.

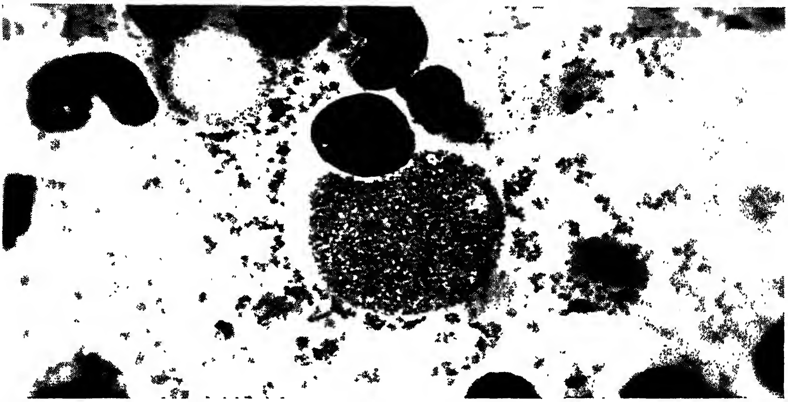


Fig. 215.—Rickettsiae of American Q fever in a smear of skin exudate. Note the macrophage packed with enormous numbers of the rickettsiae. × 1125 (Dr. F. S. Markham).

Use of Infected Insects.—Infected insects have been used as a source of rickettsiae in the preparation of vaccines for spotted fever in this country and for classic typhus in Europe. Spencer and Parker[6] have prepared suspensions containing large numbers of spotted fever rickettsiae by grinding the viscera of infected ticks. The rickettsiae are killed with phenol. A similar method has been used by Weigl[7] for the preparation of vaccine material containing the rickettsiae of classic typhus fever. The viscera of lice infected by intrarectal inoculation are ground and the killed suspensions used as a vaccine. The yield is not great.

Use of Infected Animals.—Heavy suspensions of the rickettsiae of murine typhus may be obtained from the peritoneal cavity of rats inoculated intraperitoneally five days before. The resistance of the rats is

[6] Spencer and Parker: Pub. Health Repts., 1925, *40*:2159.

[7] Weigl: Bull. Internat. d. l'Acad. Polonaise d. Sc. e. d. Lettres, 1930, p. 25.

reduced by x-ray irradiation, the injection of benzol or maintenance on a deficient diet.[8] It has also been shown by Castaneda[9] that an experimental pneumonia may be produced in rats and other animals by the intranasal inoculation of murine-typhus rickettsiae. The rickettsiae of classic typhus will produce a similar infection but very few are found in the lung tissue. Recently Castaneda and Silva[9a] have reported that multiplication of the classic type of typhus rickettsiae is much more pronounced when they are inoculated simultaneously with the murine variety. In either case enormous numbers of rickettsiae are found in the lungs and, when these are ground and centrifuged free from cellular débris, heavy suspensions of rickettsiae are obtained. Formolized suspensions prepared in this way are widely used in Mexico in prophylactic immunization against murine typhus fever.

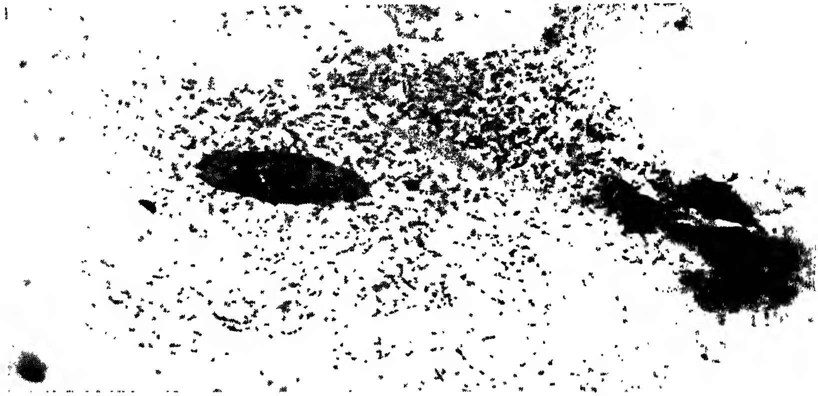


Fig. 216.—Rickettsiae of American Q fever in the yolk-sac emulsion from egg culture by Cox's method. The morphology is typical of rickettsiae in general; note the occurrence of paired microorganisms. × 1125 (Dr. F. S. Markham).

Culture.—As indicated above, the rickettsiae proliferate only in the presence of living cells. Some type of tissue culture is, therefore, obligatory in their cultivation apart from the host. Although they may be grown on the chorioallantois of the developing chick embryo only small numbers are produced. They have also been cultivated in a modified Maitland medium (minced guinea pig tunica vaginalis suspended in a mixture of serum and Tyrode's solution). Zinsser, Fitzpatrick and Wei[10] devised a culture method which makes use of a modification of the Maitland method; minced embryonic chick tissue is inoculated with rickettsiae and spread on the surface of a Tyrode solution-serum agar slant. In these cultures proliferation of the rickettsiae is not associated with active host cell multiplication;

[8] Zinsser and Castaneda: Jour. Immunol., 1931, *21*:403; Proc. Soc. Exp. Biol. Med., 1931–32, *29*:840.

[9] Castaneda: Amer. Jour. Path., 1939, *15*:467.

[9a] Castaneda and Silva: Proc. Soc. Exp. Biol. Med., 1944, *57*:80.

[10] Zinsser, Fitzpatrick and Wei: Jour. Exp. Med., 1939, *69*:179.

rather the rickettsiae appear to reach a height of proliferation as the host cells begin to die.

A different and more successful method of growing rickettsiae has been devised by Cox[11] which consists of cultivation in the yolk sac of the developing hen's egg. The rickettsiae are found in huge numbers in the contents of the sac (see Fig. 216). It was believed for a time that extracellular multiplication occurred, but it is now known that proliferation of the rickettsiae occurs entirely within the lining cells of the yolk sac and they are liberated as the cells disintegrate. This egg culture method has been widely used for the preparation of vaccines. On the death of the embryo, the embryonic tissue is suspended in phenol-formol saline and the rickettsiae washed free by centrifugation. A liter of vaccine may be prepared from 200 eggs.

Resistance.—The rickettsiae are not hardy organisms and are killed by heating to 50° C. for a few minutes, and die out, usually within a few hours, at room or incubator temperature in infectious blood and similar materials. It has been reported, however, that sealed Maitland medium cultures remain infectious even up to a few months at 37° C.[12] Rickettsiae may be preserved in infected tissues stored at $-20°$ C. or in lyophilized material for several months. Like a number of the viruses they are resistant to glycerine, and tissues suspended in glycerine and stored in the refrigerator retain infectivity for several months. In general, however, they do not survive more than a few hours apart from the host cells. Their resistance to disinfectants is of the same order as that of the more delicate bacteria.

Pathogenicity.—The rickettsiae appear to be well-established parasites of arthropods and there is good evidence for the acarinid origin of all the rickettsial diseases.[13] They are not pathogenic for the insect vectors which transmit them, except lice, and, in fact, the infection is hereditary in insects undergoing an incomplete metamorphosis, *viz.*, the ticks. They also appear to be reasonably well adapted to the animals, especially rodents, which in many instances constitute a reservoir of infection in nature. Disease may be produced experimentally in the guinea pig, however, the usual procedure in isolation of rickettsiae being the intraperitoneal inoculation of 5 ml. of blood in each of two animals.[14] A febrile attack occurs in seven to twelve days and some varieties of rickettsiae produce a characteristic orchitis or scrotal reaction. An acute fibrinous exudate is formed in the scrotal sac and many cells packed with rickettsiae may be found. The disease in the guinea pig is often not fatal, however, and this animal is much more resistant to infection than is man. Other laboratory animals are more resistant, and rats, mice, rabbits, dogs, cats, etc., may show no febrile reaction or other outward manifestation of disease though the infection has been established and in some cases, at least, persists for months.

The pathogenicity of the rickettsiae for man is often very high; certain

[11] Summarized in detail by Cox: Science, 1941, *94*:399.

[12] Nigg: Jour. Exp. Med., 1935, *61*:17.

[13] Huff: Quart. Rev. Biol., 1938, *13*:196.

[14] For a discussion of laboratory diagnostic procedures see Findlay: Proc. Roy. Soc. Med., 1941, *35*:157.

strains of spotted fever rickettsiae produce a case fatality rate of 90 per cent or more and the rate in epidemic typhus may reach 70 per cent though it is ordinarily somewhat less than half that. Others, however, produce only a relatively mild disease. Variation in virulence from strain to strain for both experimental animals and man is marked. The various types of rickettsial infection in man are considered in later sections.

The characteristic pathologic changes found in rickettsial disease result from the multiplication of the microorganisms within the endothelial cells of the small blood vessels throughout the body, and especially in the skin and brain in the case of typhus fever. It has been reported[15] that typhus rickettsiae contain a specific endotoxin but this is not established as yet.

Immunity.—Recovery from an attack of rickettsial disease usually confers a solid and lasting immunity. The development of artificial immunization procedures will be considered later but it may be noted here that the immune response is manifested by the appearance of protective antibody, of opsonins and agglutinins for the rickettsiae, and of complement-fixing antibody. The last has been of a good deal of interest recently as a differential diagnostic method and it is reported to distinguish even closely related varieties of rickettsia.[16]

The Weil-Felix Reaction.—In some rickettsial diseases agglutinins appear to certain strains of *Proteus*. This apparently anomalous response was observed by Weil and Felix[17] in 1915 and has very considerable diagnostic value. They found that a strain of *Proteus vulgaris*, which they designated X-2, was agglutinated by typhus sera, and another strain, designated X-19, was similarly agglutinated but to much higher titer; in fact, titers as high as 1:50,000 are occasionally observed in European typhus. It was further shown that the agglutinating antigen was a part of the heat-stable somatic or *O* antigen; these strains are, therefore, commonly termed OX strains. Other strains showing this immunological characteristic have been isolated since. It is of some importance that an *O* antigen be used in carrying out the agglutination because antibody to the flagellar antigen of *Proteus* occurs with some frequency in normal sera. More general application of this agglutination test, commonly known as the Weil-Felix reaction, showed that it is specific for the typhus group of fevers, sera from other rickettsial diseases agglutinating only to low titer or not at all. As pointed out elsewhere (p. 479), the phenomenon is a consequence of the presence of a common antigen in *Rickettsia prowazeki* and the X strains of *Proteus*.

The *Proteus* agglutinin is only a part of the antibody response. It is not identical with the rickettsia agglutinin for the latter appears earlier, persists longer and appears to be associated with protective antibody.[18] Furthermore, the opsonin remains in typhus serum after it has been absorbed with *Proteus*.[19]

[15] Otto and Bickhardt: Ztschr. f. Hyg. 1941, *123*:447.
[16] *Cf.* Plotz: Science, 1943, *97*:20.
[17] Weil and Felix: Wien. klin. Woch., 1916, *29*:974.
[18] *Cf.* Fitzpatrick and Hampil: Amer. Jour. Pub. Health, 1941, *31*:1301.
[19] Castaneda: Jour. Immunol., 1936, *31*:227.

Another immunological type of *Proteus*, the OX-K strains, was found[20] to be agglutinated by tsutsugamushi sera but not by typhus sera. This, coupled with the OX-19 type, makes possible the division of the rickettsial diseases into three groups, the typhus group in which OX-19 is agglutinated, the tsutsugamushi group in which OX-K is agglutinated, and the spotted fever group which is indeterminate in that neither type of *Proteus* is agglutinated to high titer. A classification of the rickettsial diseases on this basis is given in the accompanying table.

The significance of the Weil-Felix reaction is wholly unknown. The occurrence of one common antigen, or more specifically a polysaccharide haptene, in two such different microorganisms might of course occur purely by chance. The occurrence of a second common antigen, shared by another variety of *Proteus* and another group of rickettsiae, on the basis of chance alone is highly improbable. There is no evidence, however, which points

IMMUNOLOGICAL (WEIL-FELIX) GROUPING OF RICKETTSIAL DISEASES*

Immunological Group		
OX-19 OX-19 ++++ OX-2 + OX-K −	OX-K OX-19 − OX-2 − OX-K ++++	Undetermined OX-19 + OX-2 + OX-K +
Classic, European typhus Brill's disease Endemic, murine typhus of United States, Australia, Greece, Syria, Manchuria, Malay (shop typhus), India, Burma, Philippines, Hawaii, Toulon (fièvre nautique)	Tsutsugamushi of Japan, Formosa, Malay, Netherlands East Indies. "Scrub typhus" or "rural typhus" of Malay, N. E. I., French Indo-China, India, Australia Mite fever of Sumatra	Spotted fever Sao Paulo typhus Fièvre boutonneuse Fièvre exanthematique Febbre errutiva Tick fever of South Africa Kenya fever Tick typhus of India

* Modified from Felix: Brit. Med. Jour., 1942, ii:497.

to any other connection between *Proteus* and the rickettsiae. There is, for example, no association between the X strains and typhus; X strains are found in diseases other than typhus, and *Proteus* strains other than X strains are found in typhus. *Proteus* infections, of course, often result in the formation of specific agglutinins; Dammin and Billings,[21] for example, have found that the sera of persons infected with *Pr. vulgaris*, *Pr. mirabilis* or *Pseudomonas pyocyanea* will agglutinate OX-K strains of *Proteus* to a high titer. Such persons might be said to give a positive Weil-Felix reaction.

Classification.—The rickettsiae appear to be intermediate between the bacteria and the viruses. Most of the pathogenic bacteria that are obligate parasites in that they are incapable of a successful saprophytic existence are readily cultivable on artificial media, in some cases very simple media. In the host they may be actually outside the tissues proper as in

[20] Fletcher and Lesslar: Bull. Inst. Med. Res., F. M. S., No. 2, 1925.
[21] Dammin and Billings: Jour. Immunol., 1942, *44*:251.

RICKETTSIAL

	Rickettsia		Disease in Man
	Name	Synonyms	
Typhus group	*Rickettsia prowazeki* var. *prowazeki*......	....	European typhus, classic typhus, epidemic typhus, louse-borne typhus Brill's disease
	Rickettsia prowazeki var. *mooseri*........	*Rickettsia muricola*	Murine typhus, endemic typhus, tick typhus Shop typhus Toulon typhus (fièvre nautique) Moscow typhus Manchurian typhus Tabardillo (Mexican typhus)
Spotted fever group	*Rickettsia rickettsii*...	*Dermacentroxenus rickettsii*	Rocky Mountain spotted fever, spotted fever
	Rickettsia rickettsii...	*Rickettsia rickettsii* var. *brasiliensis*	São Paulo typhus Tobia fever (Colombia)
	Rickettsia rickettsii...	*Rickettsia rickettsii* var. *conori* *Rickettsia conori*	Fièvre boutonneuse (Marseilles fever) Kenya typhus
	Rickettsia rickettsii...	*Rickettsia rickettsii* var. *pijperi*	African tick fever
Tsutsugamushi group	*Rickettsia orientalis*...	*Rickettsia tsutsugamushi* *Rickettsia nipponica* *Rickettsia akamushi*	Tsutsugamushi disease, Kedani fever, Japanese flood fever (Japan) Rural typhus, scrub typhus (Malaya) Mite fever (Sumatra)
Unrelated group	*Rickettsia diaporica*...	*Rickettsia burneti*	Q fever American Q fever Nine-mile fever
	Rickettsia pediculi....	*Rickettsia quintana* *Rickettsia wolhynica* *Rickettsia weigli*	Trench fever, Wolhynian fever

the case of the dysentery bacilli and the cholera vibrio, but in most instances they are tissue parasites. With *Pasteurella tularensis* and *Bartonella bacilliformis* an intracellular existence is the rule but both can be cultivated on lifeless, though complex, media. The rickettsiae, while morphologically resembling bacteria, are intracellular parasites that cannot be grown in the absence of living cells and in this respect they resemble the viruses. In fact, the elementary bodies of certain of the larger viruses, such as psittacosis virus, are morphologically indistinguishable from small rickettsiae; conversely, rickettsiae found in the lungs of infected animals closely resemble the forms described in the developmental cycle of the psittacosis virus[22] (see also p. 822). The resemblance is also apparent in other respects for the pneumonitis produced by *Rickettsia diaporica* of American Q fever

[22] Begg, Fulton and van den Ende: Jour. Path. Bact., 1944, *56*:109.

DISEASES OF MAN

Weil-Felix Type	Scrotal Reaction	Vectors	Vertebrate Reservoir	Geographical Distribution
OX–19	+	*Pediculus humanus*	Man	Europe, Asia, South America
OX–19	+++	*Xenopsylla cheopis* *Xenopsylla astia* *Polypax spinulosa*	Rat, squirrel, shrew	World-wide
Indeterminate	+++	*Dermacentor andersoni, D. variabilis,* *Hemaphysalis leporis-palustris* Possibly *Amblyomma americanum, A. cajennense, Dermacentor occidentalis, D. parumapertus, Ornithodorus parkeri, Rhipicephalus sanguineus*	Small rodents, rabbits, goats	Continental United States
Indeterminate	+++	*Amblyomma cajennense, A. striatum, A. brasiliense*	Dog, opossum	Brazil Colombia
Indeterminate	+++	*Rhipicephalus sanguineus* *Amblyomma nebraeum*	Dog Small rodents?	Mediterranean, South Africa
Indeterminate		*Amblyomma hebraeum, Rhipicephalus appendiculatus, Hemaphysalis leachi, Boophilus decoloratus*	Small rodents? Dog?	South Africa, Abyssinia
OX–K	–	*Trombicula akamushi,* *Trombicula deliensis,* *Trombicula schuffneri* (Malay, Sumatra)	*Microtus montebelli* (Japan) *Mus concolor,* *Mus diardii* (Malay, Sumatra)	Japan Sumatra, Malay
Indeterminate	–	*Hemaphysalis humerosa* *Dermacentor andersoni, D. occidentalis, Amblyomma americanum*	Bandicoot Small rodents?	Australia, western United States
?	?	*Pediculus humanus*	Man?	Europe

very closely resembles that produced by the psittacosis virus. Clearly, then, it is possible to set up a continuous series of parasitic types in which the ricksettsiae occupy an intermediate position.

The question of the formal classification of the rickettsiae is another matter. As yet too little is known of the interrelationships of these microorganisms to permit subdivision on a basis other than that of expediency. Although it is clear that differences of generic status exist, it is generally agreed that formal classification is premature at present.[23] The generic name *Rickettsia* has been generally adopted except in the case of the spotted fever rickettsia, which was named *Dermacentroxenus rickettsii.* It is hardly justifiable to single out this one type and here this microorganism will be designated *Rickettsia rickettsii.*

[23] See, however, Philip: Amer. Jour. Hyg., 1943, *37*:301.

spinulosa.[30] The disease may also be transmitted by the human louse and, when a case occurs by transmission from the rat in a community where there are lice in abundance, murine typhus may become an epidemic louse-borne infection. Such epidemics occur from time to time in Mexico. Murine typhus occurs with considerable frequency in this country, and approximately 20,000 cases were officially recorded in 1932–1941. Georgia is the chief endemic center with about 1000 cases per year, the other southern states contributing the remainder. Isolated cases are reported from widely scattered regions including Boston, New York, St. Louis, Cleveland, etc., and the disease is apparently slowly spreading.[31] Control is essentially a matter of rat control.[32]

Murine typhus also occurs elsewhere in the world and is known by various names. The urban type occurring in Malaya or "shop typhus," the mild form of the disease in the Mediterranean region known as *Toulon typhus*, and other infections such as *Moscow typhus*, *Manchurian typhus* and the *Red Fever of the Congo* are all murine typhus identical or nearly so with the type found in the United States and Mexico.

Clinically murine typhus does not differ appreciably from the classic European type. Both may exist in endemic and epidemic form and the differentiation between endemic (murine) and epidemic (European) typhus is not sound. It is not infrequently said that the European disease is the more fatal, but this does not appear to be true; both forms are equally fatal in epidemic form and relatively mild in the endemic form.

Differences between the two varieties of *R. prowazeki* are demonstrable but not great. The murine rickettsiae produce a necrotic scrotal reaction in guinea pigs, while the European variety does not; the murine variety may be carried indefinitely in mice without alteration, but the European variety tends to degenerate; the rickettsial pneumonia and the intraperitoneal multiplication in rats with the production of enormous numbers of rickettsiae noted earlier may be produced by the murine variety but not by the European type alone. There is a slight immunological difference also, for, while recovery from either infection results in a solid and lasting immunity to both, murine vaccines protect against murine infection but only incompletely against infection with the rickettsiae of European typhus. Plotz[33] has found that the two may be differentiated by complement fixation.

The Weil-Felix Reaction in the Typhus Fevers.—As indicated earlier, the agglutination of *Proteus* OX-19 strains is a valuable adjunct in the diagnosis of these affections. The agglutinin appears relatively early, reaching a titer as high as 1:400 by the end of the first week and rising to 1:1600 or above by the end of the second week. It drops thereafter, falling to 1:400 or less by the eighth or ninth week after onset. There is, of course, great variation in the observed titers.

[30] *Cf.* Dyer *et al.:* Pub. Health Repts., 1931, *46:*334, 470, 1869, 2481; Mooser, Castaneda and Zinsser: Jour. Exp. Med., 1931, *54:*567.

[31] Topping and Dyer: Amer. Jour. Trop Med., 1943, *23:*37.

[32] *Cf.* Eskey: Pub. Health Repts., 1943, *58:*631.

[33] Plotz: Science, 1943, *97:*20.

Normal serum from persons living in endemic localities often shows an agglutinin titer of 1:50 and titers as low as this are regarded as negative, 1:100 as suggestive, and 1:200 as diagnostic. The Weil-Felix reaction shows an anamnestic response in a number of febrile diseases, especially in typhoid fever. In typhoid fever, however, the titer of the Weil-Felix reaction does not continue to rise after the first week, whereas typhoid bacillus agglutinins continue to rise in titer.

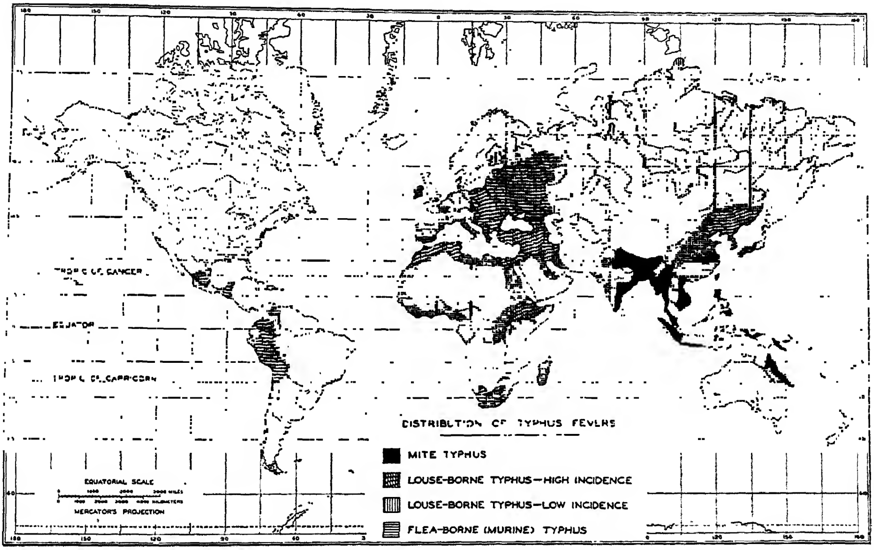


Fig. 217.—The world-wide distribution of typhus fevers, including classic or European typhus, murine typhus and mite typhus of the Far East. Redrawn from maps prepared by Army Medical Intelligence, 1943. (Based on Goode *Base Map* No. 201M. By permission of the University of Chicago Press.)

Though classed as an indeterminate immunologic type, spotted fever frequently shows a considerable agglutinin titer for *Proteus* and differentiation from murine typhus may be difficult. Differential diagnosis may be established by complement fixation with rickettsial antigen.[34] Microscopic or macroscopic agglutination of rickettsial antigen appears to be practical but has not as yet been generally applied for diagnostic purposes.[35]

THE SPOTTED FEVERS

The rickettsial diseases which make up the spotted fever group are considerably more heterogeneous than those of the typhus fever group. Several of them are but poorly understood as yet. All are, however, transmitted by ticks, although some differ immunologically and clinically from Rocky Mountain spotted fever.

[34] Bengtson and Topping: Amer. Jour. Pub. Health, 1942, *32*:48.

[35] *Cf.* Van Rooyen and Bearcroft: Edinburgh Med. Jour., 1943, *50*:257.

Rocky Mountain Spotted Fever.—Clinically Rocky Mountain spotted fever resembles typhus fever; the rash is generally more extensive and the nervous symptoms may be more pronounced, but in areas where both diseases prevail it is exceedingly difficult to distinguish them on clinical grounds alone.

The disease has been known for many years in the Rocky Mountain region in states such as Idaho, Montana, Wyoming, Oregon, and Washington. In 1930, however, it was found[36] in the eastern part of the United States in the South Atlantic states such as Maryland, Virginia, West Virginia, and North Carolina. Although the disease is not limited to these regions, of the 2190 cases reported from 1933 to 1937, 65.5 per cent were from the Mountain and Pacific states and 27.4 per cent from the South Atlantic group; the two areas combined accounted for 93 per cent of the total cases reported in the country.

It is customary to speak of two types of the disease, an eastern type and a western type. Both are, however, immunologically identical and are caused by the same rickettsia, *Rickettsia rickettsii* (*Dermacentroxenus rickettsii*), which, in contrast to the typhus rickettsia, is found within the nucleus of invaded cells. Both are transmitted by ticks, but by different species. The wood tick, *Dermacentor andersoni*, is the vector of the disease in the western states, and the dog tick, *Dermacentor variabilis*, transmits the disease in the Atlantic region. The rabbit tick, *Hemaphysalis leporis-palustris*, disseminates the infection in the animal population. *Amblyomma americanum*, *A. cayennense*, *Dermacentor occidentalis*, *Rhipicephalus sanguineus*, *Dermacentor parumapterus* and *Ornithodorus parkeri* are able to transmit the disease experimentally and are to be regarded as potential vectors. The disease is hereditary, *i.e.*, is transmitted from one generation to the next, in *D. andersoni*, but whether it is hereditary in the dog tick is not clear. In the first instance, then, there need be no animal reservoir of infection. In the second, the dog may serve as a temporary reservoir of infection. The epidemiology of the eastern variety, however, is not well understood.

The case fatality of spotted fever is highly variable. In Idaho it has been about 4 per cent, in western Montana about 20 per cent, and in certain regions of the Bitterroot Valley it is as high as 90 per cent. Over the country as a whole, however, the rate is 18 to 19 per cent. It is generally said that the disease is milder in the Atlantic region than in the Mountain states, but over a period of years there is little difference in case fatality; for the period of 1933–37 the case fatality in the Mountain and Pacific states was 19.4 per cent and for the South Atlantic states 18.1 per cent.[37]

Spotted fever was transmitted to guinea pigs and monkeys by Ricketts.[38] Guinea pigs show a febrile reaction, the spleen is enlarged, and the necrotic scrotal reaction occurs. Castaneda and Silva[39] have found that there is a slight cross protection between spotted fever and typhus.

[36] Rumreich, Dyer and Badger: Pub. Health Repts., 1931, *46:*463, 470.
[37] For further detail see Topping: Pub. Health Repts., 1941, *56:*1699.
[38] Ricketts: Jour. Inf. Dis., 1907, *4:*141.
[39] Castaneda and Silva: Jour. Immunol., 1941, *42:*1.

A solid immunity appears upon recovery from the disease, and active immunization with the vaccine prepared from the viscera of infected ticks may be carried out.[40] This vaccine confers full protection against mild strains but only occasional protection against the highly virulent strains of rickettsiae. The duration of the immunity probably does not exceed one year and annual revaccination is necessary. There is some evidence that persons who are successively vaccinated are better protected than those receiving but a single dose. Some fifteen years' experience with this vaccine has been summarized by Parker.[41] It has remarkable keeping qualities; three samples stored for twelve to fourteen years in the refrigerator were found to retain full potency.[42]

The therapeutic use of immune serum has been a distinct possibility; recent experimental trial of immune rabbit serum in human cases of spotted fever has suggested that it has definite therapeutic value.[43]

São Paulo Typhus.—The disease termed São Paulo typhus is not typhus but is immunologically identical with spotted fever. The symptoms are very similar to those of spotted fever, and the case fatality is very high, possibly 70 per cent. It also produces the necrotic scrotal lesions in the guinea pig. It is transmitted by the tick *Amblyomma cajennense.*

Fièvre boutonneuse (Marseilles fever) is a disease prevalent in the region of the Mediterranean and Black seas. The animal reservoir of infection is the dog, and the disease is transmitted by a hereditary tick vector, *Rhipicephalus sanguineus.* The disease is immunologically very closely related to spotted fever but may be differentiated by complement fixation[44] and differs somewhat in its clinical aspects. It runs a considerably milder course and the case fatality is only 1 to 2 per cent. There is also a primary sore and an adenitis of the regional lymph nodes.

Kenya fever is also immunologically identical with spotted fever and is transmitted by the tick *Rhipicephalus sanguineus.* It differs clinically, however, from fièvre boutonneuse in that there is no primary sore and no adenitis.

South African tick fever is a rickettsial disease, sometimes transmitted by *Rhipicephalus sanguineus* but more commonly by the larvae of *Amblyomma hebraeum,* a parasite of wild rodents. Clinically the disease is very similar to fièvre boutonneuse in that there is a primary sore and adenitis.[45]

"Q" Fever.—A rickettsial infection designated as "Q" fever has been described by Derrick and by Burnet and Freeman[46] in Australia which is immunologically distinct from spotted fever although sera from patients do not agglutinate *Proteus* X strains. Infection in man has been found for the most part in abattoir workers. The bandicoot (*Isoodon torosus*) ap-

[40] *Cf.* Spencer: Jour. Inf. Dis., 1929, *44*:257.
[41] Parker: Amer. Jour. Trop. Med., 1941, *21*:369.
[42] Parker and Steinhaus: Pub. Health Repts., 1943, *58*:230.
[43] Topping: Pub. Health Repts., 1943, *58*:757.
[44] Plotz, Reagan and Wertman: Proc. Soc. Exp. Biol. Med., 1944, *55*:173.
[45] Derrick: Med. Jour. Australia, 1937, *2*:281.
[46] Burnet and Freeman: Med. Jour. Australia, 1937, *2*:299; *ibid.*, 1938, *1*:296; *ibid.*, 1938, *2*:1114; *ibid.*, 1939, *1*:11.

pears to harbor the disease in nature and a number of other rodents and marsupials are susceptible to experimental infection.[47] It is transmitted among animals and possibly also to man by the tick *Hemaphysalis humerosa.* Guinea pigs and other laboratory animals may be infected experimentally. The scrotal reaction is not produced in the guinea pig. The rickettsia has been designated *Rickettsia burneti* by Derrick.[48]

The filter-passing rickettsia isolated by Davis in Montana produces a disease in guinea pigs which has been termed *nine-mile fever.*[49] The rickettsia has been designated *Rickettsia diaporica* by Cox.[50] A few laboratory infections have occurred, including a small outbreak involving fifteen persons.[51] and a naturally acquired infection has been reported.[52] Wood ticks (*D. andersoni*) transmit the infection under experimental conditions and the infection in the tick is hereditary.

It has been found[53] that nine-mile fever and "Q" fever are immunologically identical, or practically so, although the American strains are somewhat more virulent. It has been suggested, therefore, that nine-mile fever be called American "Q" fever.

Whether South African tick fever and "Q" fever should be included in the spotted fever group is problematical. Their immunological dissimilarity separates them, but their clinical similarities and failure to agglutinate *Proteus* X strains suggest their inclusion in this group.

Bullis Fever (Lone Star Fever, Tick Fever).—This disease was described by Woodland, McDowell and Richards[53a] who observed it in soldiers stationed at Camp Bullis near Houston, Texas. About 1000 cases had been observed through 1943. The onset is abrupt, with fever lasting four to fourteen days and falling by lysis; lymphadenopathy and pronounced leucopenia are observed, together with a maculopapular rash on the trunk in severe cases. The case fatality rate is negligible.

Livesay and Pollard[53b] established the infectious agent in guinea pigs by the intraperitoneal inoculation of patients' blood; a low-grade febrile reaction of forty-eight hours' duration was produced on the ninth to tenth day. Peritoneal scrapings and spleen smears showed rickettsia-like microorganisms, and similar forms have been found in biopsy specimens from enlarged lymph nodes of patients. Convalescent sera fixed complement specifically with mouse spleen antigen and the disease appears to be immunologically distinct from Q fever and murine typhus.[53c] The Weil-Felix reaction is negative.

[47] Derrick, Smith and Brows: Australian Jour. Exp. Biol. Med. Sci., 1940, *18:*409.
[48] Derrick: Med. Jour. Australia, 1939, *1:*14.
[49] *Cf.* the detailed studies of Parker: Pub. Health Repts., 1938, *53:*2267; Cox: *ibid.*, 1938, *53:*2270; Cox: *ibid.*, *54:*1822, 2171; Parker, Kohls, Cox and Davis: *ibid.*, 1939, *54:*1482; Davis: *ibid.*, 1939, *54:*2219.
[50] Cox: Pub. Health Repts., 1939, *54:*1822.
[51] Dyer, Topping and Bengston: Pub. Health Repts., 1940, *55:*1945.
[52] Zemp: Jour. Amer. Med. Assn., 1943, *121:*838.
[53] Dyer: Pub. Health Repts., 1939, *54:*1229; Bengston: Pub. Health Repts., 1941, *56:*272.
[53a] Woodland, McDowell and Richards: Jour. Amer. Med. Assn., 1943, *122:*1156.
[53b] Livesay and Pollard: Amer. Jour. Trop. Med., 1943, *23:*475.
[53c] *Ibid.*, 1944, *24:*281.

It seems probable that the infection is transmitted by the tick, *Amblyomma americanum*, and there is some evidence that deer and rabbits may be infected.

THE TSUTSUGAMUSHI GROUP

The three rickettsial diseases that make up the tsutsugamushi group closely resemble one another in that they are immunologically identical or nearly so, they are all transmitted by mites, the animal reservoirs of infection are rats or mice, and *Proteus* OX–K is agglutinated by patients' sera.

Tsutsugamushi Disease (Japanese Flood Fever, *Kedani* Fever).—This rickettsial disease is very similar to spotted fever in its restriction to certain localities (uncultivated areas along the banks of rivers) and in the clinical picture presented. It differs, however, in that there is a primary sore and adenitis, as in fièvre boutonneuse. The rickettsia has been variously termed *R. nipponica*, *R. orientalis*, *R. tsutsugamushi* and *R. akamushi*. The disease is very difficult to produce in experimental animals; guinea pigs are resistant to infection. In man, however, it is a severe disease and the case fatality is around 40 per cent. About 400 cases are reported each year in Japan.

The disease is transmitted by the larva of *Trombicula akamushi* and the animal reservoir of infection is the field mouse, *Microtus montebelli*.[54]

Mite Fever of Sumatra.—This disease is identical both clinically and immunologically with tsutsugamushi disease in Japan and is transmitted by the larva of *Trombicula deliensis*, a mite very closely related to *T. akamushi*. The animal reservoirs of infection appear to be the house rat, *Mus concolor*, and the field rat, *Mus diardii*.

"Rural Typhus" or "Scrub Typhus" of Malaya.—This disease is to be differentiated from the "shop typhus" of Malaya, which is murine typhus. It is a considerably milder disease than tsutsugamushi disease, with which it appears to be immunologically identical, and there is no initial sore and bubo. The disease is transmitted by both *Trombicula akamushi* and *T. deliensis*. The animal reservoir of infection is the rat. Guinea pigs are highly resistant to inoculation but may be infected if they are previously made scorbutic. Monkeys may be infected by intradermal inoculation.[55]

TRENCH FEVER

In the course of the First World War a specific infection became known under the name of trench fever or Wolhynian fever. It is said to have caused almost one-third of all the illness in some of the armies in northern France and occurred also in Mesopotamia and Saloniki. It appeared again in the Second World War in the German Army in Russia.[56] It has a long incubation period (six to twenty-two days); the most constant symptom

[54] The earlier Japanese investigations are discussed by Kawamura: *Studies on Tsutsugamushi Disease*. University of Cincinnati. 1926.

[55] *Cf.* the recent work on this disease of Lewthwaite and Savoor: Jour. Path. Bact., 1936, *42*:23; Trans. Roy. Soc. Trop. Med. and Hyg., 1935–36, *29*:561; Brit. Jour. Exp. Path., 1936, *17*:208, 448.

[56] Jacobi: Muench. Med. Woch., 1942, *89*:615.

is pain in the legs; the fever is often high and of the relapsing type. Recovery is the rule. The disease can be transmitted to healthy men by the intravenous injection of whole blood taken from patients up to the fifty-first day of the disease.

Natural transmission is chiefly, if not solely, through the body louse. The bites of infective lice appear to be the main vector in producing trench fever, but the virus is also present in the excreta and may enter through abrasions in the skin caused by scratching. The virus is present in the urine of patients and is said to remain active for a long time in dry louse feces and dried urine.

The studies of Arkwright, Bacot and Duncan[57] established the fact that in most cases lice or the excreta of lice that had been proved by inoculation to contain the virus contained large numbers of rickettsiae. Bacot, who became accidently infected with trench fever, had for over two years previously been feeding upon his person a stock of lice known to be free from rickettsiae. Two days after the onset of his attack, he resumed the feeding of these lice, and rickettsiae were observed eight days afterward in enormous numbers in their excreta. Infection of the lice with rickettsiae continued possibly for as long as three months after the disappearance of all symptoms. Nothing, however, is known certainly about the occurrence of rickettsiae in the bodies of patients with trench fever. Further evidence is necessary to establish the causal relation of *Rickettsia pediculi* (also called *R. quintana*) to trench fever, although there is a strong presumption that such a connection obtains. The disease was not reproduced in experimental animals.

RICKETTSIAL DISEASE OF ANIMALS

In addition to the rickettsial diseases of man which often exist in nature in animal reservoirs of infection, other rickettsial infections occur in animals but not in man.

Heartwater Disease.—This is a highly fatal disease of cattle, sheep and goats in South Africa. The causative organism, *Rickettsia ruminantium*, was described by Cowdry.[58] It is somewhat different morphologically from the typhus and spotted fever rickettsiae in that rod-shaped forms are rare, most of the organisms being rounded or elliptical and occasionally sharply curved. It has not been cultivated and laboratory animals do not appear to be susceptible to infection though successful passage of the disease in ferrets has been reported.[59]

In infected animals the disease resembles the human rickettsia infections in that the vascular endothelium is parasitized, but differs in that large volumes of fluid accumulate in the pericardium, pleura and peritoneal cavity—hence the name heartwater. It is transmitted by *Amblyomma hebraeum*. Infection persists for some time after clinical recovery and immunity to reinfection is of a low order.

[57] Arkwright, Bacot and Duncan: Jour. Hyg., 1919–20, *18*:76.
[58] Cowdry: Jour. Exp. Med., 1925, *42*:231, 253.
[59] Mason and Alexander: Jour. South African Vet. Med. Assn., 1940, *11*:98.

Other Animal Rickettsiae.—Three other apparently closely related rickettsiae have been described in disease of animals.[60] *Rickettsia ovina* was found in sheep, *Rickettsia canis* in dogs and *Rickettsia bovis* in cattle. These rickettsiae are morphologically indistinguishable from one another and all are characterized by invasion of the circulating monocytes. They appear to be transmitted by ticks of the genera *Rhipicephalus* and *Hyalomma.*

[60] Donatien and Lestoquard: Arch. Inst. Pasteur (Algiers), 1937, *15:*142.

CHAPTER 36

THE FILTERABLE VIRUSES

By F. B. Gordon, Ph.D., M.D.*

The existence of agents, apparently living but sufficiently small to be invisible under the microscope, and capable of passing filters which retain ordinary bacteria, was indicated by the work of Iwanowski in 1892[1] and of Beijerinck,[2] who found that bacteria-free filtrates of the juice of tobacco plants affected with tobacco mosiac would produce the disease in healthy plants. The similar filterability of the infectious agent of foot-and-mouth disease was soon demonstrated by Löffler and Frosch.[3] Since the beginning of the present century a great variety of infectious diseases of man, of lower animals and of plants have been shown to be due to similar agents, among them smallpox and the pock diseases of lower animals, rabies, poliomyelitis, yellow fever and a host of others. Reports of newly discovered filterable agents appear at frequent intervals and the list of so-called virus[4] diseases has steadily grown longer.

Opposite is a list of the more important virus diseases of mammals (including man) and fowls. Bacteriophage, which may be regarded as a virus disease of bacteria, is discussed in Chapter 38.

The pathogenic agents grouped under the head of filterable viruses defy precise definition, though in the aggregate they exhibit properties which are highly characteristic. As yet no descriptive statement sufficiently specific to have value and not requiring considerable qualification has been made concerning these agents. Though termed filterable, some viruses pass through bacteria-proof filters with difficulty or not at all, and although most viruses are not visible with ordinary microscopic technique—hence the term *ultramicroscopic virus*—some of the larger ones may be observed by special methods of illumination or in certain stained preparations. It is not infrequently stated that active immunity to the virus disease is usually solid, but in some instances immunity is quite transient. Ability to survive in 50 per cent glycerin is a property of many but not all viruses, and some bacteria also possess this property. Without exception, however, no virus has as yet been cultivated in the absence of

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[1] *Cf.* Iwanowski: Centralbl. f. Bakt., II Abt., 1899, *5*:250.

[2] Beijerinck: Centralbl. f. Bakt., II Abt., 1899, *5*:27.

[3] Löffler and Frosch: Centralbl. f. Bakt., I Abt., 1898, *23*:371.

[4] The word "virus" has been used as a general term covering different types of infectious agents, especially when one did not wish to be specific concerning the nature of the agent. It was natural that the term "filterable virus" should be used for the disease agents of unknown nature which could be set off from the known agents by their ability to pass bacterial filters. At present the term "virus," unless another meaning is obvious, is usually synonymous with "filterable virus."

living tissue cells, on even the richest of the artificial media suitable for cultivation of the most fastidious bacteria. There is also a lack of substantial evidence for an independent metabolism on the part of the viruses. Although all reports are not in complete agreement on this point, experiments[5] with purified vaccinia virus, to which special weight should be given, have yielded negative results. It may be noted, however, that some evidence of a respiratory mechanism has been detected in purified preparations of this virus (p. 794).

The failure to define these agents satisfactorily is a consequence of a lack of precise knowledge concerning them, for, although there is some information regarding their nature, many of them are, like the antigens and antibodies, known mostly in terms of what they do rather than what they are. It is quite possible also that viruses are a heterogeneous group of entities, taxonomically speaking, having in common only (1) size below a certain maximum (actually the range of sizes is enormous, Fig. 224), and (2) habits of strict cellular parasitism, regardless of how this arose.

COMMON VIRUS DISEASES[6]

Variola	Poliomyelitis
Vaccinia	Rabies
Influenza	Pock diseases of animals
Common cold	Fibroma and myxoma of rabbits
Measles	Papilloma of rabbits
Mumps	Ectromelia of mice
Varicella	Pseudorabies (cattle)
Herpes zoster	Rinderpest (cattle plague)
Molluscum contagiosum	Foot-and-mouth disease (cattle)
Warts	Louping ill (sheep)
Psittacosis	Swine influenza
Ornithosis	Hog cholera
Lymphogranuloma venereum	Vesicular stomatitis (horse)
Trachoma	African horse sickness
Lymphocytic choriomeningitis	Equine infectious anemia
Yellow fever	Sarcomas of fowls
Rift valley fever	Fowl plague
Dengue fever	Fowl pox
Phlebotomus fever	Newcastle disease of fowls
Encephalitis (various types)	Infectious laryngotracheitis of fowls

In spite of the obvious and perhaps inevitable lack of exact definition of these agents, and present inability to differentiate them sharply from the bacteria in terms of any single characteristic, the filterable viruses in the aggregate attain a certain individuality which more than adequately justifies the unique position in which they are generally placed.

Cytotropism.—The intimate and obligatory relation between the viruses and the cells of the host is the most important element in the definition of this group of agents, though it does not serve to set them off sharply from the rickettsiae; some species of bacteria and protozoa are also intimately associated with host cells.

[5] Parker and Smythe: Jour. Exp. Med., 1937, *65*:109.

[6] This list makes no pretense of being exhaustive and omits entirely the virus diseases of amphibians, fish, insects and plants. For a more complete list see McKinley: Puerto Rico Jour. Pub. Health Trop. Med., 1934, *9*:299.

Tissue Predilection.—The viruses show, in most instances, a marked predilection for certain tissues. A large group of viruses, including variola and vaccinia, molluscum contagiosum, trachoma and the pock diseases of lower animals, produce primary lesions of the epithelial surfaces of the body; viruses showing such affinities for the skin are termed *dermotropic*. Another large group, designated as *neurotropic* viruses, including rabies, poliomyelitis, encephalitis and others, produces lesions primarily in the central nervous system. Still other viruses, *e. g.*, virus of yellow fever, which attack the abdominal or thoracic viscera or produce signs indicative of generalized infection, are termed *viscerotropic*. The virus of influenza and certain others found in the lungs have been called *pneumotropic*. The term *pantropic* is used to indicate affinities for many tissues; some prefer to reserve this term for viruses which are able to parasitize derivatives of all three primitive embryonic layers.

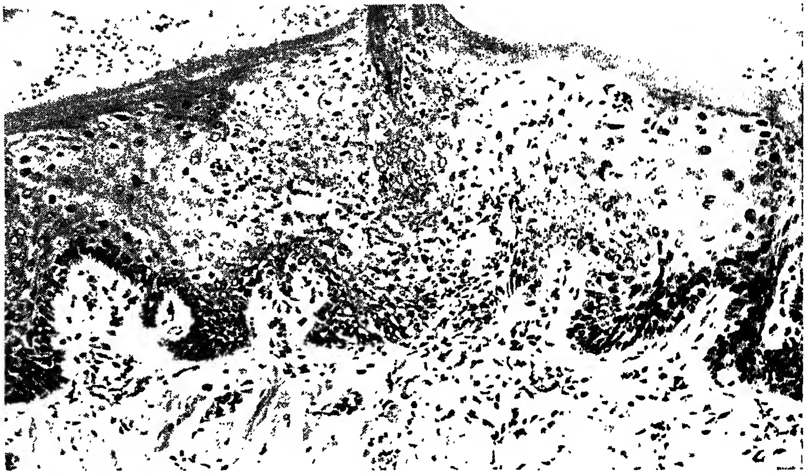

Fig. 218.—Foot-and-mouth disease. The section is from the tongue of a guinea pig and shows an early necrotic lesion of the epithelium. × 200 (obtained through the courtesy of Dr. I. A. Galloway).

Such tissue affinities are not, however, absolute. The adjectives applied are indicative of the most apparent site of attack; secondary infection of other tissues not infrequently occurs, more commonly with some viruses than with others. As will appear, these tissue affinities may sometimes be altered by appropriate manipulation.

Effect upon the Host Cells.—In general, the pathology of the virus diseases runs the gamut of the possible types of tissue injury.[7] Necrotic and degenerative changes, acute and chronic inflammation and hyperplasia are all represented in varying combinations and locations. In most cases, it is generally believed, the effects are produced by actual invasion of the

[7] *Cf.* Rivers: Amer. Jour. Path., 1928, *4*:91.

susceptible cells by the viruses, soluble toxins playing no part. A toxic action of the virus of lymphogranuloma venereum and others of this group (p. 819) has, however, been demonstrated. Heavy emulsions of this virus apparently act in the same manner as a bacterial endotoxin and antiserum will neutralize the effect of the toxin.

Evidences of proliferation and degeneration are present in most virus infections, and the nature of the lesion is determined by the relative degree of each. Virus infections may be arranged in an orderly series according to the relative significance of these two factors.[8] At the one extreme are those viruses which produce degeneration and necrosis almost exclusively. Such, for example, are foot-and-mouth disease (Fig. 218) and the pock diseases. Severe necrosis of the liver cells is produced by the viruses of yellow fever (Fig. 233), Rift Valley fever and ectromelia. A greater

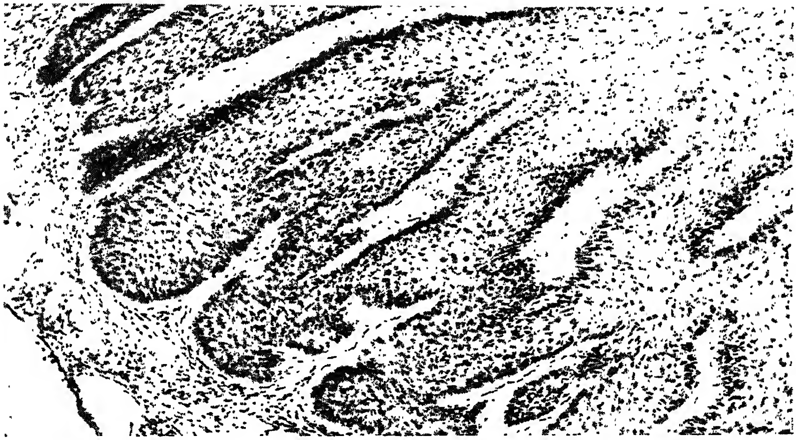

Fig. 219.—Rabbit papilloma. Hyperplasia of epithelial tissue is evident. × 125 (obtained through the courtesy of Dr. W. J. Purdy).

amount of proliferation is seen in other instances, however; in fowlpox, for example, sometimes called epithelioma contagiosum, necrosis does not begin until after considerable hyperplasia has occurred. Viruses at the other extreme produce primarily a proliferation, and degenerative and necrotic changes play little or no part in determining the character of the lesion. Such is the case, for example, in rabbit fibroma, rabbit papilloma (Fig. 219), human warts and the filterable sarcomas of fowls.

In connection with the proliferative tendencies manifested in certain virus diseases, it may be noted that the filterable fowl sarcomas, of which a number have been described, have all the characteristics of malignant tumors.[9] They may be composed of very anaplastic cells, invade tissue rapidly, metastasize, and kill the host. The inoculation of susceptible fowls with cell-free filtrates of emulsified tumor tissue is followed by the

[8] *Cf.* Andrewes: Lancet, 1934, *ii*:63.

[9] *Cf.* the following reviews: Claude and Murphy: Physiol. Rev., 1933, *13*:246; Rous: *Virus Diseases*, Cornell University Press. Ithaca. 1943. pp. 147–170.

appearance of a similar tumor. Similar transmission by cell-free filtrates of fowl tumors induced by tar has been reported in a few instances.[10] Rabbit papilloma, an infectious wart of cottontail rabbits, is due to a filterable agent. When transmitted to domestic rabbits these warts generally progress to true cancer and there is serological evidence that the papilloma virus persists in the malignant tumor, although it is not demonstrable by direct inoculation. It appears that the virus plays a significant, though not necessarily a primary, rôle in the production of the cancer.[11] Numerous attempts have been made to demonstrate filterable agents in malignant mammalian tumors, but the results have been almost uniformly negative.

Obvious evidences of tissue disturbance need not follow infection with filterable viruses, and *latent infections* with these agents occur just as they do with the pathogenic bacteria. A certain proportion of some stocks of white mice, for example, are healthy carriers of the virus of lymphocytic choriomeningitis (p. 827), and when the host-parasite equilibrium is disturbed by the intracerebral inoculation of sterile broth, an acute attack of disease ensues. Similarly, the virus known as Virus III may be latent in the testes of normal rabbits but produces an orchitis when testicular tissue is serially transferred. Many of the plant viruses also produce such a latent infection; their presence may be demonstrated by passage to a susceptible strain of plant in which the manifestations of the disease will be evident. The practical significance of such latent virus infections to the study of experimental virus diseases is obvious.

Inclusion Bodies.—The presence of the so-called inclusion bodies within the affected cell, either in the cytoplasm or in the nucleus, is perhaps another manifestation of the cytotropic character of the filterable viruses. These bodies are of varying size and appearance and are readily demonstrable in histological sections by differential staining. They are found in most, but apparently not all, virus diseases and are not found in bacterial infections, even with those microorganisms which invade the cells.

Intracytoplasmic inclusion bodies are found associated with vaccinia (Fig. 220), molluscum contagiosum, variola, rabies (Figs. 221 and 238), trachoma, fowlpox, infectious myxomatosis of rabbits, the viruses of the psittacosis-lymphogranuloma group (Fig. 232), and certain others. They exhibit a considerable degree of individuality, and their recognition is of practical significance in the diagnosis of some virus diseases. One of the best known inclusion bodies in this connection is the Negri body (Fig. 221) found in rabies.

Intranuclear inclusion bodies are of two types. Type A, found in cells in the lesions of yellow fever, herpes (Fig. 229), varicella, B virus disease, Virus III of rabbits (Fig. 222), and others, consists of an acidophilic mass which disrupts the normal structure of the nucleus and may occupy a large fraction of the space within it. The accumulation of chromatin at the mar-

[10] McIntosh and Selbie: Brit. Jour. Exp. Path., 1939, *20*:49.

[11] *Cf.* Rous, Kidd and Beard: Jour. Exp. Med., 1936, *64*:385; Rous, Beard and Kidd: *ibid.*, 1936, *64*:401; Kidd: *ibid.*, 1938, *68*:703, 725, 737; Kidd: *ibid.*, 1940, *71*:335, 351.

Fig. 220.—Guarnieri bodies, the intracytoplasmic inclusion bodies of vaccinia. The arrows point to two inclusions among many in the infected corneal epithelium of a rabbit's eye. Giemsa stain; × 650.

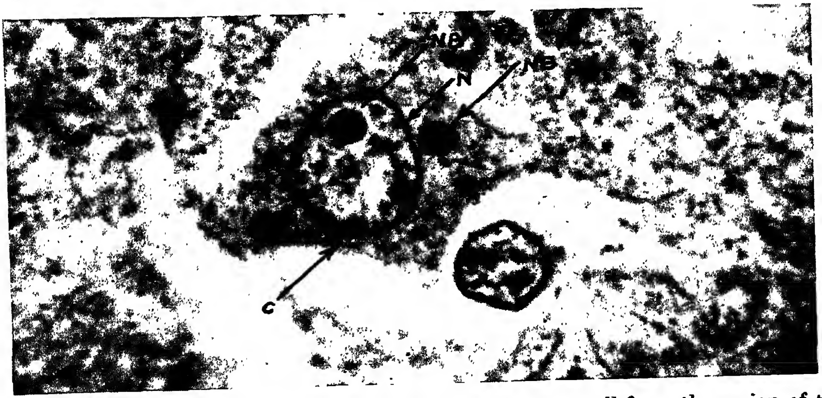

Fig. 221.—Negri bodies in human brain. This large nerve cell from the region of the hippocampus contained seven Negri bodies; five of various sizes are visible in the photograph. C = edge of cytoplasm of cell; N = edge of nucleus; NB = Negri bodies. Alzheimer's stain, reduced from × 2575.

gin of the nucleus as seen in Fig. 222 is characteristic. Type B, described in Borna disease (an encephalomyelitis of horses), poliomyelitis, and a few others, consists of smaller bodies which characteristically are found within an otherwise normal-appearing nucleus. In contrast to the cytoplasmic inclusions, the nuclear inclusions do not have a morphology characteristic for the infection in which they are found. In fact, their significance in relation to virus infection is not at all clear, for they may be produced by other means as well. Inclusion bodies resembling those of yellow fever, for example, have been found in the cells of the liver following severe burns. In other cases the inoculation of chemical agents, *e. g.*, the sub-

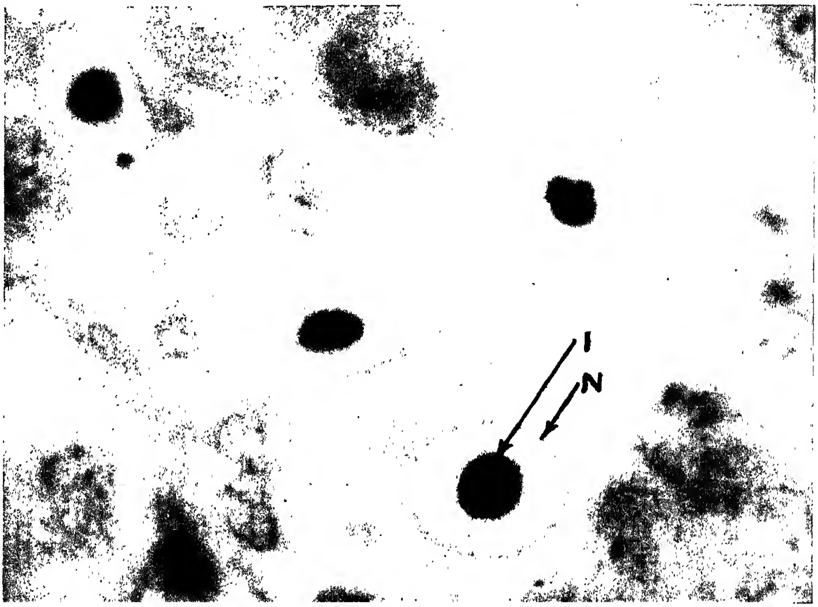

Fig. 222.—Intranuclear (Type A) inclusion bodies of Virus III. I = inclusion body; N = marginated chromatin at the periphery of nucleus. This is from a section of infected rabbit testis. Giemsa stain; × 2550. (Obtained through the courtesy of C. H. Andrewes.)

cutaneous inoculation of guinea pigs with aluminum hydroxide, alundum or fresh or autoclaved brain tissue, has resulted in the appearance of nuclear inclusions in the macrophages.[12] The presence of intranuclear inclusion bodies, then, is not necessarily indicative of virus infection.

The Nature of Inclusion Bodies.—Inclusion bodies have been regarded by some as products of cellular degeneration accumulated within the affected cell as, it may be noted, some of the nuclear inclusions not associated with virus infection may readily be. Some of the early workers suggested that these bodies were protozoan in nature and represented a stage in

[12] Olitsky and Harford: Proc. Soc. Exp. Biol. Med., 1938, *38*:92.

a complex life cycle of the microorganism. Von Prowazek[13] considered the inclusion body to be composed of a mass of small virus particles, which he called *elementary bodies*, surrounded by a mantle of amorphous material produced by the cell in reaction to the virus. On this basis he suggested that viruses should be called *Chlamydozoa* or "cloak animals." Since then it has been found that a number of intracytoplasmic inclusion bodies are essentially of this nature (Figs. 223 and 232). The evidence that the elementary body is the infectious particle of virus is presented below. It will suffice to state here that intracytoplasmic inclusion bodies, in some instances at least, may be regarded as "colonies" of virus within the cell.

The Physical Characters of Viruses.—Although Beijerinck postulated the presence of a *contagium vivum fluidum* in infectious plant juice,

Fig. 223.—Left, partially disrupted inclusion bodies of ectromelia from a culture on the chorio-allantois of a duck egg. Darkfield illumination × 600. Right, a portion of the same field under higher magnification showing the constituent elementary bodies. Darkfield illumination × 2200. (From Himmelweit: Brit. Jour. Exp. Path., 1938, *19*:108. Reproduced through the courtesy of the author and publishers.)

subsequent studies with animal viruses indicated that these agents are particulate in nature. Minute corpuscular elements, early noted by Borrel[14] in the lesions of fowlpox, were regarded by him as the causal agent of the disease. Von Prowazek and Paschen[15] described similar bodies in material from variola and vaccine lymph in which they were especially numerous. Similar elements have since been demonstrated in a number of virus infections and are known as elementary bodies (Fig. 228). Mention has already been made of their occurrence within inclusion bodies. Woodruff and Goodpasture[16] supplied the first direct evidence of the relation of inclusion body and elementary body to virus. They succeeded in freeing the inclusion bodies of fowlpox from other cellular material and demonstrated

[13] Von Prowazek: Arb. Gesundheitsamt, Berlin, 1905, *22*:535.
[14] Borrel: Compt. Rend. Soc. Biol., 1904, *57*:642.
[15] Paschen: Münch. med. Wchnschr., 1906, *49*:2391.
[16] *Cf.* Goodpasture: Harvey Lectures, 1929–30, p. 77.

the infectivity of a single inclusion body. Furthermore, they showed that the inclusion body was composed of numerous elementary bodies and that infectivity was associated with the latter. In the case of certain other viruses the presence of active virus in filtrates or centrifugates of infectious emulsions is directly correlated with the presence of elementary bodies. The size of the virus as determined by indirect means (see below) agrees well with the observed size of the elementary bodies. Furthermore, these bodies are agglutinated in specific immune serum. The intimate association between virus activity and the elementary bodies has become increasingly clear, and it is highly probable that the elementary bodies are, in fact, the virus. There is a large number of viruses, however, in which elementary bodies have not been demonstrated and whose size would seem to preclude their being rendered visible by present methods.

Size.—The viruses show, as a group, an enormous range in size (Fig. 224). The smallest have a size, estimated by differential filtration, of approximately 10 mμ, although in the case of the virus of polioymelitis, one of the smallest, this may be the shorter dimension of a rod-like particle. The largest appear to be spherical particles with diameters as great as 250 mμ.

Filtration.—As noted earlier, some viruses readily pass diatomaceous earth or unglazed porcelain filter candles, while others are filterable only with difficulty or not at all. Such filters do not, however, act as mechanical sieves, and it is well known that the electrical charge on the particles (like the bacteria, the viruses are negatively charged at neutrality) and that on the filter and, as a corollary, the nature of the suspending medium, all play a part in determining filterability.[17] The ability to pass through such filters is, therefore, related to particle size in only a crude way.

The use of graded collodion membranes or ultrafilters, frequently termed *gradocol membranes*, has added a quantitative element to filtration experiments, for these filters, though to some degree affected by charge, act much like mechanical sieves. Such membranes were originally introduced by Bechold[18] for the study of colloids. Elford[19] and others[20] have prepared membranes of known porosity[21] by drying solutions of collodion of varying concentration in thin sheets or by impregnating some carrier such as filter paper. The use of a series of such membranes of graded porosity allows a relatively accurate determination of particle size. Such filtration of viruses has been highly successful and much of our information concerning the size of viruses has been obtained in this way.

Ultracentrifugation.—The size of virus particles also determines their rate of sedimentation in a centrifugal field. The high speeds required to throw down the minute virus particles necessitate special apparatus and the technique has been termed ultracentrifugation. Several methods have been employed. One, adapted from an earlier method of Schlesinger and

[17] Kramer: Jour. Inf. Dis., 1927, *40:*343.

[18] Bechold: Ztschr.. f. phys Chem., 1907, *60:*257; *ibid.*, 1908, *64:*32.

[19] Elford: Jour. Path. Bact., 1931, *34:*505; Proc. Roy. Soc., Ser. B, 1933, *112:*384.

[20] Allisbaugh and Hyde: Amer. Jour. Hyg., 1935, *21:64*; Bauer and Hughes: Jour. Gen. Physiol., 1934–35, *18:*143.

[21] The determination of porosity is based upon Poiseuille's law (*cf.* Cox and Hyde: Amer. Jour. Hyg., 1932, *16:*667) and is probably not strictly accurate.

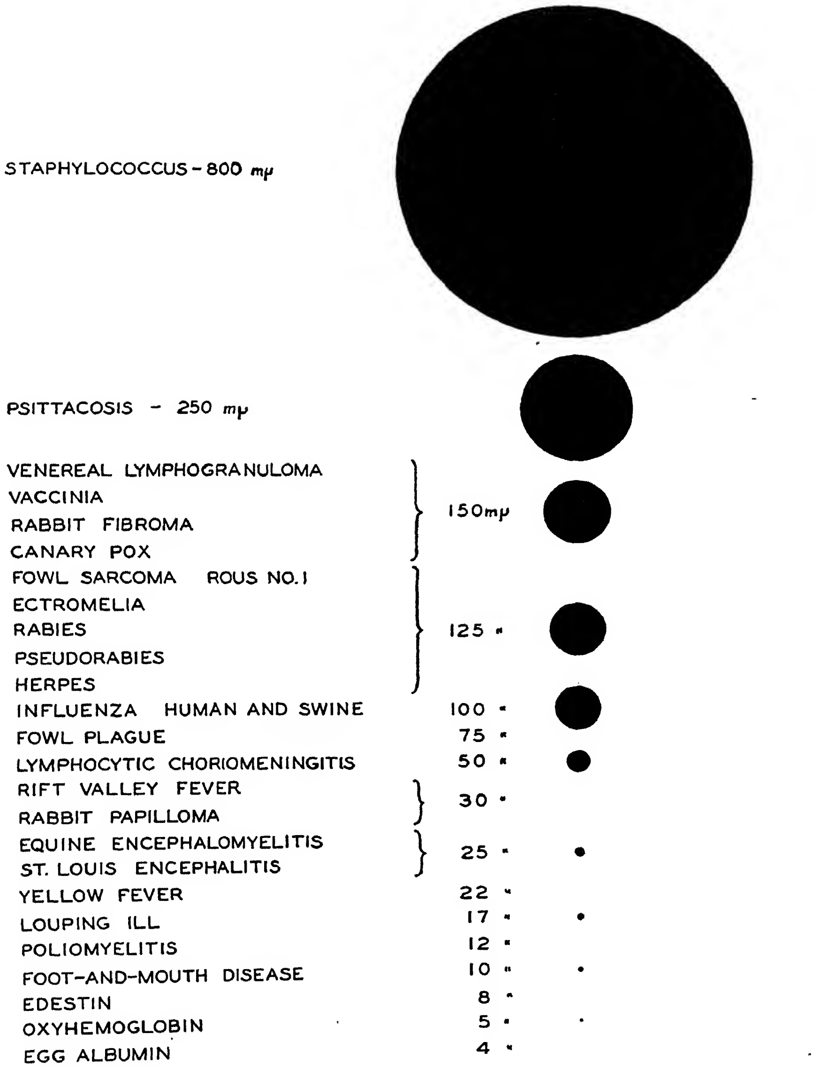

Fig. 224.—Chart showing the relative sizes of a staphylococcus, representative viruses, and several protein molecules. The virus particles are assumed to be spherical and the measurements represent approximate means between limits obtained by ultrafiltration. The diameter of the virus particle of lymphocytic choriomeningitis, for example, is found to lie between 40 and 60 mμ; it is indicated here as 50 mμ. Since these values were obtained estimations of sizes by other methods have in some cases given different values. Adapted from the data of Elford, Bauer and others.

modified by McIntosh and Selbie[22] and by Elford,[23] involves the titration of virus before and after centrifugation. In the capillary-tube method of Elford, a capillary tube sealed at one end is inverted in a liquid reservoir; a ten-fold drop in titer of the material within the tube (*i.e.*, nine-tenths of the virus is removed) indicates that the mean distance a particle has sedimented during the run is nine-tenths the length of the tube. From this figure the rate of sedimentation, and in turn the size of the particle, may be determined. Centrifugation of virus particles through a density gradient with subsequent test for concentration of virus at various levels has also been used.

Another method of ultracentrifugation is an adaptation of that devised by Svedberg for the study of sedimentation rates of proteins. The high-speed centrifuge is so arranged that ultraviolet light may be directed through the cell while the machine is in motion. Exposure of photographic plates at intervals allows sedimentation within the cell to be followed be-

Fig. 225.—Sedimentation of elementary bodies of vaccinia: *a* and *b* indicate the upper and lower edges respectively of the sedimenting boundary; the distance between constitutes the spread. From Pickels and Smadel: Jour. Exp. Med., 1938, *68*:583. (Reproduced through the courtesy of the authors and publisher.)

cause of differential absorption of the light by various concentrations of sedimenting material. With homogeneous suspensions, *e.g.*, elementary bodies, a definite sedimenting boundary is visible (Fig. 225), and the rate at which the boundary moves represents the sedimentation rate of the virus particles. A relatively high degree of purification of the virus is usually a necessary preliminary to this type of observation.

An essential preliminary to the determination of particle size by sedimentation rate is knowledge of the density of the particle; other variables such as viscosity of the suspending medium, etc., can, of course, be controlled. Methods of estimating the density of virus particles are not entirely satisfactory, and there is considerable variation in the figures from different sources. The density of vaccinia virus has been reported as 1.16, but this can be changed by inducing different degrees of hydration of the particle by employing suspending media of different osmotic pressures.

[22] McIntosh and Selbie: Brit. Jour. Exp. Path., 1937, *18*:162.
[23] Elford: Brit. Jour. Exp. Path., 1936, *17*:399.

There is evidence[24] that the density of some smaller viruses is nearer that of proteins, 1.30 to 1.35. A recent estimation of the density of influenza virus[25] gives 1.104. The values for particle size given for sedimentation rates approximate fairly well those obtained by filtration through gradocol membranes.

Microscopy.—The stained elementary bodies of some of the larger viruses, including psittacosis (Fig. 231), vaccinia, fowlpox, canarypox, ectromelia and infectious myxoma, are resolved by the ordinary microscope with visible light. Silver-impregnation methods (Fig. 228), Victoria blue, or methylene blue and azur stains are suitable.[26] The size of the elementary bodies is, of course, distorted owing to the deposition of stain.

The larger elementary bodies may be seen in the ordinary darkfield microscope, though resolution is no better than with transmitted light. Incident oblique illumination, giving an effect similar to that of a darkfield condenser, has also been used (Fig. 223), either alone or in conjunction with fluorescent dyes such as primulin.[27]

The resolving powers of the microscope are limited, as pointed out elsewhere (p. 40), by the wave length of visible light, and may therefore be extended through the use of shorter wave lengths such as unltraviolet. By this method, using quartz lenses, Barnard[28] has been able to secure photomicrographs of the elementary bodies of some of the larger viruses such as vaccinia, canarypox and ectromelia. The size of these bodies agrees well with the values obtained by filtration experiments.

The electron microscope (p. 41) has been employed for studying the appearance of purified preparations of a number of viruses. In general the determinations of size by this method have agreed with those estimated by other techniques.

Shape.—A necessary assumption in the determination of particle size by filtration experiments is that of a spherical shape of the virus particle. That such an assumption is justified in many instances is indicated by direct microscopical evidence. Himmelweit's photomicrographs (Fig. 223), for example, show that the viruses observed are coccoid in shape, and stained elementary bodies are likewise round or oval. Nevertheless, examination of the "coccoid" elementary bodies of vaccinia by electron microscopy (Fig. 226) reveals that these bodies, when thoroughly washed, appear to be brick-shaped. Interesting evidence of internal structure is also apparent. Influenza virus is said to consist of rounded or ovoid particles as viewed with the electron microscope and the same is true of the virus of equine encephalomyelitis. But certain other viruses have been shown by this and other methods to be cylindrical or filamentous in shape. Tobacco mosaic virus consists of particles 15 by 280 mμ and there is partial evidence that poliomyelitis virus may have a similar shape.

[24] Tang, Elford and Galloway: Brit. Jour. Exp. Path., 1937, *18*:269.
[25] Sharp, *et al.*: Science, 1944, *100*:151.
[26] *Cf.* Gildemeister, Haagen and Waldmann: *Handbuch der Viruskrankheiten*, Chap. 2. Gustave Fischer. Jena. 1939.
[27] *Cf.* Himmelweit: Lancet, 1937, *ii*:444.
[28] *Cf.* Barnard and Elford: Proc. Roy. Soc., Ser. B, 1931, *109*:360.

The Effect of Physical and Chemical Agents.—Almost all experiments to determine the effect of physical and chemical agents upon viruses have necessarily been performed using impure virus preparations containing tissue elements and other extraneous organic matter. Although probably not giving correct values for pure virus, such experiments have obvious practical value. In some instances suspensions of elementary bodies or other purified preparations have given more reliable evidence concerning the resistance of the virus itself.

Heat.—In fluid emulsions most viruses are inactivated by heat at about the same times and temperatures as vegetative bacterial cells; the rates of destruction of foot-and-mouth disease virus and *Bacterium typhosum* are similar between 50° and 60° C. Some viruses appear to be somewhat more resistant, however. Glycerinated vaccine lymph is said to resist

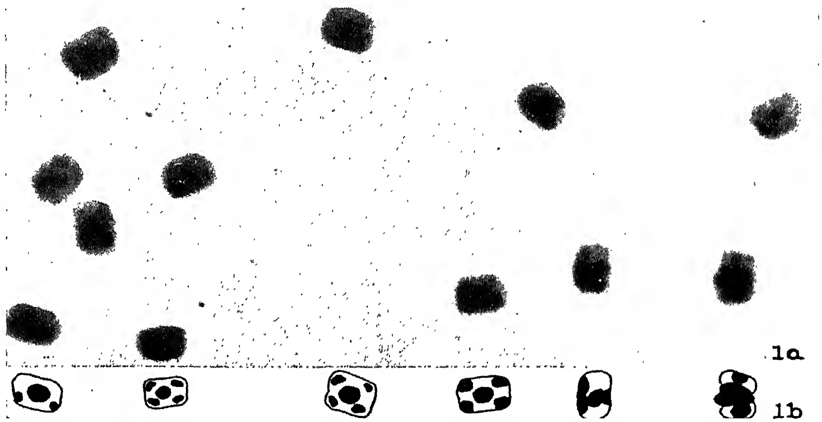

Fig. 226.—*1a*, Structure of purified elementary bodies of vaccinia as revealed by the electron microscope. *1b*, Schematic representation of certain of the virus particles shown above. Magnification 7100×4 (From Green, Anderson and Smadel: Jour. Exp. Med., 1942, *75*:651. Reproduced through the courtesy of the authors and publishers).

55° C. for thirty minutes and hog-cholera virus in blood resists the same temperature for two hours. Vaccinia virus in the dried state is reported to resist 100° C. for five to ten minutes. Plant viruses vary considerably in their resistance to moist heat; thermal death points range from 42° to 90° C. for ten-minute exposures.

Extreme cold exerts a preservative action, and many viruses may be frozen, dried, and stored with little loss of activity. Virus-containing tissues may be frozen and stored at temperatures obtainable with dry ice (−76° C.) for relatively long periods of time without deterioration.

Radiation.—Direct sunlight inactivates viruses readily; presumably the ultraviolet portion of the spectrum is involved, for exopsure to ultraviolet light results in a rapid inactivation. Fowlpox and vaccine virus are said to be more resistant than bacteria to ultraviolet light, the latter virus surviving

in vaccine lymph when adventitious bacteria are killed. Although infectivity of a virus emulsion is easily destroyed by ultraviolet light, selection of the correct exposure time leaves the antigenicity of the preparation at a high level. This has been exploited experimentally in the preparation of several immunizing preparations (pp. 838, 851, 855).

Desiccation.—Most viruses remain active for long periods in the dry state. Crusts from variolous patients are said to remain infective for years, and artificially dried vaccine virus has been found active after four months at room temperature.

Chemical Agents.—It is commonly stated that viruses are somewhat more resistant to the phenols than are bacteria, and this disinfectant is used to reduce the number of bacteria in vaccine lymph, leaving the virus active. Tests with vaccinia virus and others with purified influenza virus suggest that inactivation occurs readily with oxidizing agents (potassium permanganate, iodine), while foot-and-mouth-disease virus is resistant. Soaps and other detergents will inactivate influenza virus. Reducing agents, cysteine in particular, have been found to retard deterioration of virus preparations. A number of viruses are resistant to ether (vaccinia, poliomyelitis, rabies), but St. Louis encephalitis virus is quickly inactivated. Several viruses are inactivated by bile salts and urea, and many are inactivated by treatment with formalin. Few viruses have been tested in a pure state against chemical or other agents and the results with crude emulsions can be indicative of generalities only. Tests with purified influenza virus,[29] however, have confirmed in general the ideas gained in previous studies.

The Chemical Composition of Viruses.—Only a few viruses have been purified and prepared in sufficient quantity to allow a reasonably accurate chemical analysis. Some of the plant viruses have been the most thoroughly investigated because of the tremendous amount of virus activity present in the diseased tissues.

Tobacco-Mosaic Virus.[30]—Apparently pure proteins, intimately associated with virus activity, have been prepared from the juice of diseased tobacco plants by ammonium sulfate precipitation and by high-speed centrifugation. The protein appears to be the same irrespective of the species of the plant host, and forms needle-like "crystals" which are, in fact, paracrystals oriented in two dimensions only. These have more the properties of natural fibers and may be visualized as bundles of rodlike molecules lying parallel and overlapping one another in the direction of their long axis. The molecular weight of this substance is high; it has been estimated to be 40 million. Repeated recrystallization results in no loss of virus activity and, in general, inactivation coincides with destruction or alteration of the protein, which is now considered to be identical with the virus. Chemical analysis indicates that the substance is a nucleoprotein and a number of the amino acids present have been identified. It has been

[29] Knight and Stanley: Jour. Exp. Med., 1944, *79*:291.

[30] *Cf.* the following reviews: Stanley: Physiol. Rev., 1939, *19*:524; *Virus Diseases*, Cornell University Press. Ithaca. 1943, pp. 35–59; Wyckoff: Ergeb. d. Enzymforsch., 1939, *8*:1; McFarlane: Biol. Rev., 1939, *14*:223.

found that strain differences within the tobacco mosaic virus group are reflected in quantitative and qualitative differences in the amino acid makeup of the molecule, with a preservation of the fundamental structural pattern.

Similar "heavy proteins" have been found associated with the viruses of a number of plant diseases, and these likewise form paracrystals. The protein associated with the virus of bushy stunt of tomatoes, however, shows no evidence of asymmetry and forms true crystals.

Substances of high molecular weight and homogeneous as determined by sedimentation in the ultracentrifuge have been demonstrated in purified preparations of several animal viruses also. In some cases the evidence is good that these represent virus, in others the nature of the sedimentable substance is obscure. One of the reasons for uncertainty is that similar sedimentable substances have been demonstrated in certain normal tissues. In those cases where the chemical composition of the virus can be reasonably ascertained it has been found that the virus activity is closely associated with nucleoprotein, as is the case with the plant viruses. In some cases a lipoid is also present in the complex. None of the animal virus proteins isolated, however, forms either paracrystals or true crystals.

Vaccinia Virus.—Studies[31] upon a virus of relatively large particle size (vaccinia) have shown that the composition is complex, the analysis of dried elementary bodies giving values for protein, fat and carbohydrates not unlike those observed with bacteria. Nucleoprotein is present and nucleic acid (thymus type) constitutes about 6 per cent of the elementary body and lipoid a like amount. Copper and a flavin-adenine-dinucleotide have been demonstrated to be integral parts of the elementary body, suggesting that an incomplete respiratory mechanism may be present. Biotin also is present in purified preparations of this virus.

Cultivation of Viruses.[32]—As noted above, the filterable viruses require the presence of living host cells for their proliferation; they cannot be cultivated on lifeless media. They are, like the spirochete of syphilis, often maintained in the laboratory by serial passage through susceptible animals. It may be noted that a number of viruses persist in infectious tissue for long periods of time when stored in the cold. *In vitro* culture methods have been devised, however, in which the virus is grown in the presence of living host cells either in tissue culture or in the developing hen's egg.

Tissue Culture.—Many of the viruses may be propagated in tissue cultures such as those of Carrel in which there is proliferation of the tissue itself. Proliferation of the host cells is, however, apparently not essential, and viruses such as vaccinia have been cultivated by the Maitland technique[33] in which fresh minced hen's kidney and hen's serum are incubated in a Carrel flask. Li and Rivers[34] have used minced chick embryo tissue in

[31] *Cf.* the review by Rives: *Virus Diseases*, Cornell University Press. Ithaca. 1943, pp. 1–31.

[32] *Cf.* the critical summary of culture methods by Sanders: Arch. Path., 1939, *28:*541.

[33] Maitland and Maitland: Lancet, 1928, *ii:*596.

[34] Li and Rivers: Jour. Exp. Med., 1930, *52:*465.

Tyrode's solution with or without serum. Under such circumstances there is little or no proliferation of the tissue cells, but their viability persists for some days. By this technique many viruses, including those of vaccinia, yellow fever, vesicular stomatitis, equine encephalomyelitis, St. Louis encephalitis, pseudorabies, influenza and others, have been cultivated. Other types of embryonic tissue may be used; the viruses of rabies and St. Louis encephalitis have been grown in association with mouse-embryo brain.

Egg Culture.[35]—It was found by Woodruff and Goodpasture[36] that viruses may be cultivated on the chorioallantoic membrane of the developing chick embryo. Some gross alteration of the membrane is usually produced, and when the dilution of inoculated virus is suitable, isolated opaque lesions (consisting of hyperplasia, necrosis and inflammation) called "pocks" may be found (Fig. 227). Some viruses, when placed upon the chorioallantoic membrane, invade the body of the embryo; louping-ill virus may be found in the brain and liver, for instance, and St. Louis en-

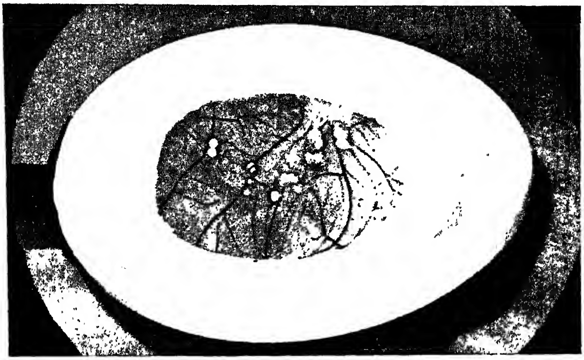

Fig. 227.—Lesions produced by ectromelia virus on the chorioallantoic membrane of the developing hen's egg. (From Himmelweit: Brit. Jour. Exp. Path., 1938, *19*:108. Reproduced through the courtesy of the author and publishers.)

cephalitis virus invades the chick brain. With repeated passages many viruses gain in virulence and kill the embryo. Influenza virus and others will infect the respiratory tract of the embryo when inoculated into the amniotic cavity.[37] An important advance in the study of influenza virus was made when it was discovered that a good growth of the virus occurs after inoculation into the allantoic cavity.[38] The virus is recoverable from allantoic fluid in a relatively pure state.

The yolk sac of the fertile egg was found by Cox to be especially suitable for cultivation of rickettsiae (p. 765) and the technique has proved to be excellent for growing viruses of the psittacosis-lymphogranuloma group (p. 819).

[35] *Cf.* the monograph by Burnet: Med. Res. Council (Great Britain), Spec. Rept. Ser., No. 220, 1936.
[36] Woodruff and Goodpasture: Amer. Jour. Path., 1931, *7*:209.
[37] Burnet: Brit. Jour. Exp. Path., 1940, *21*:147.
[38] Burnet: Austral. Jour. Exp. Biol. and Med. Sci., 1941, *19*:291.

Variation.[39]—Adaptive response to environmental conditions, a reaction associated with living organisms, is exhibited by many, perhaps all, viruses. Although observable characters are few, alterations in the virulence, or pathogenicity, and immunological character of the viruses are well known.

As in the case of the bacteria, strains of a given virus are found in nature which differ from one another in virulence and antigenic character. The so-called benign and malignant varieties of variola (p. 803) differ considerably in pathogenicity, as evidenced by case-fatality rates. Similarly, the eastern and western varieties of equine encephalomyelitis differ, the former being the more virulent. These two varieties show immunological differences, and strains of other viruses, such as those of poliomyelitis, influenza A and others, are also immunologically different in minor respects.

Adaptation.—Alterations in virulence and other characters may occur under the controlled conditions of the laboratory. One of the best known examples is seen in the conversion of rabies "street virus" to "fixed virus." This is accomplished by serial intracerebral passage in the rabbit and results in a gradually increasing virulence as evidenced by shortened incubation periods until the limit is reached. The virulence then remains constant at that level. During this process the strain loses certain characteristics including its ability to infect the central nervous system after subcutaneous inoculation, and its ability to produce Negri bodies.

Alterations in pathogenicity as expressed in changed tissue affinities are frequently observed. Passage of the "normal" (dermotropic) strain of vaccinia through rabbit testis and then to rabbit brain has given rise to a neurotropic strain of enhanced virulence which produces an encephalitis in rabbits. Yellow fever virus, in nature both neurotropic and viscerotropic, has been observed to lose its viscerotropism during serial passage in mouse brain. Cultivation may produce similar changes in some instances; a neurotropic strain of the influenza virus has been produced[40] by cultivation of the naturally occurring pneumotropic form in the chick embryo, producing first a hemorrhagic encephalitis of the embryo and, on serial passage of the culture virus through mouse brain, an encephalitis in mice.

"*Mutation.*"—The changes described above are perhaps to be regarded as gradual adaptive responses to the environment, possibly analogous to similar changes in bacteria and the *Dauermodifikationen* of higher forms. Sudden alterations, termed by some "mutations," are also observed. Such, for example, is the sudden, apparently spontaneous, variation of the rabbit fibroma virus to give, not the tumor-like proliferative fibrous lesion, but a lesion characterized chiefly by inflammation.[41] Most variant strains, including this, retain their antigenic relationship to the parent strain. In some cases, however, immunological character may be altered; such variants of the foot-and-mouth-disease virus have been observed.[42] An induced

[39] *Cf.* the review by Findlay: Jour. Roy. Microscop. Soc., 1936, *56:*213.
[40] Stuart-Harris: Lancet, 1939, *i:*497.
[41] Andrewes and Shope: Jour. Exp. Med., 1936, *63:*157.
[42] Daubney: Jour. Comp. Path., 1934, *47:*259.

conversion, superficially analogous to the transmutation of pneumococcus types (p. 155), of rabbit fibroma virus to the immunologically related virus of infectious myxoma has been reported.[43]

Reversion.—The alterations in a virus arising, presumably, as an adaptive response to changed environmental conditions, are, in some instances at least, partially or completely reversible. The "fixed virus" of rabies, for example, has been observed to revert partially to the characteristics of "street virus" by serial passage in the sciatic nerve of rabbits,[44] and viscerotropism has been restored to a neurotropic strain of yellow-fever virus by intrahepatic passage in the monkey.[39] This reversion of the yellow-fever virus has also been found to occur apparently spontaneously,[45] a matter of some practical importance since a variant strain of this type is used for human immunization.

The interpretation of the phenomena of variation in the viruses presents almost precisely the same difficulties as the interpretation of similar phenomena in bacteria. This matter has been discussed at length elsewhere (p. 156) and need not be considered here.

Immunity to Virus Diseases.[46]—Recovery from some virus diseases, *e. g.* measles and yellow fever, is accompanied by the development of solid and lasting immunity, but others, such as influenza, produce only a temporary resistance. There is no reason for doubting that immunity in virus diseases is of the same general nature as that seen in other infections, but the intracellular position of the virus introduces other factors which deserve special attention.

Active immunity may be produced artificially in a number of ways, including inoculation with virulent virus, with attenuated virus, or with virus treated with bactericidal agents. The inoculation of virulent virus, either by an unnatural route or in conjunction with antiserum injected separately or mixed with the virus, often produces a solid immunity comparing favorably with that resulting from clinical infection. Intramuscular inoculation of fully virulent psittacosis virus has been used for the immunization of laboratory workers; the normal portal of entry is probably the respiratory tract and disease does not result from the artificial inoculation. Hogs are usually immunized against hog cholera by the simultaneous injection of virus-containing blood in one groin and serum in the other. The inoculation of virulent virus, is, however, an inherently dangerous procedure and one that is seldom applied to man though frequently used in the laboratory.

Another method of immunization is the inoculation of a strain of virus attenuated by adaptation to another host. Vaccinia is perhaps to be regarded as variola attenuated by animal passage, and the inoculation of man with vaccine virus provides a solid immunity to smallpox. Similarly, rabies prophylactic is rabies virus attenuated for man by adaptation to

[43] Berry and Dedrick: Jour. Bact., 1936, *31*:50.

[44] Nicolau and Kopciowska: Compt. Rend. Soc. Biol., 1935, *119*:140.

[45] Findlay and MacCallum: Brit. Jour. Exp. Path., 1938, *19*:384.

[46] *Cf.* the reviews of Bedson: Proc. Roy. Soc. Med., 1937, *31*:59; Burnet, Keogh and Lush: Australian Jour. Exp. Biol. Med., 1937, *15*:227; 3rd lecture in Rivers: *Viruses and Virus Diseases,* Stanford Univ. Publ. Ser. Med. Sci., 1939, Vol. 4, No. 1.

the rabbit, and recently developed methods for active immunization against yellow fever make use of a virus strain adapted to tissue culture.

The problem of producing effective immunizing preparations in which the virus is entirely non-infective is complex. At one time it was thought that such preparations would not produce the immune state, a belief probably based on the fact that it was impossible to introduce enough virus antigen into the host to induce a detectable immune state except by injecting active virus and letting it multiply there. Since methods have been discovered for producing large amounts of virus, such as by cultivation in fertile eggs, it has been possible to produce effective antigens in which the infectivity has been entirely destroyed. Care must be exercised in the selection of an inactivating agent to avoid using one that destroys the antigenicity as well as the infectivity. Formalin and ultra violet radiation have been most satisfactory to date for preparing viral vaccines; the former is used in equine encephalomyelitis and influenza vaccines, and the latter has proven of value experimentally with several viruses.

There is some evidence that the lasting immunity following a virus disease, when it does occur, is due to an infection-immunity (p. 685). The virus of infectious equine anemia, for example, has been found in the blood of a horse as long as fifteen years after recovery,[47] submaxillary gland virus persists as an inapparent infection in guinea pigs, vaccinia virus has been recovered from rabbits as long as 252 days after infection, and viruses persist indefinitely in plants as an inapparent infection. Other work has failed to show a correlation between persistence of *detectable* vaccinia virus and immunity.[48] That virus may persist in at least some cases in association with the immune state would appear, however, to be definitely established. Obviously a vaccine made with an inactive virus cannot be expected to produce lasting immunity if the latter is dependent upon persistence of living virus.

Humoral antibodies are developed as a consequence of immunization with the viruses as evidenced by the protective properties of immune serum. The *serum neutralization test,* the most commonly used immunological reaction in the study of the virus diseases, consists of the inoculation of a susceptible animal with a mixture of virus and serum; immune serum will protect against many lethal doses of virus. The nature of the protective process is not clear.[49]

The antibody content of antiviral sera may be demonstrated in other ways. The agglutination of elementary bodies by immune serum may be observed in the case of the larger viruses both microscopically and macroscopically in the same manner as the agglutination of bacteria.[50] Precipitin tests in vaccinia and variola were early described, and in the case of vaccinia the flocculation has been found to be due both to a true precipitation

[47] *Cf.* Watson: *Genetics of Viruses Pathogenic to Animals*, 2nd paper in *The Genetics of Pathogenic Organisms*. Science Press. 1940.

[48] *Cf.* Pearce: Jour. Inf. Dis., 1940, *66:*130; Morgan and Olitsky: Jour. Immunol., 1940, *39:*1.

[49] *Cf.* the discussion by Salaman: Brit. Jour. Exp. Path., 1938, *19:*192.

[50] Craigie and Wishart: Brit. Jour. Exp. Path., 1934, *15:*390.

of soluble antigen and to an agglutination of elementary bodies.[51] Precipitin tests with specific immune sera have been described with the soluble antigens of lymphocytic choriomeningitis and of infectious myxoma. The complement-fixation test has been reported to be successful in a variety of virus diseases, including vaccinia, lymphocytic choriomeningitis, equine encephalomyelitis, yellow fever, influenza, and others. Of the *in vitro* serological tests, complement fixation is perhaps best adapted to the study of the virus infections since relatively small amounts of antigen are required.

The contribution of humoral antibody to immunity against virus infections is variable from one disease to another. There is reason to believe that antibody is ineffective against intracellular virus, and it would seem to follow that once a virus becomes established in the tissues considerable multiplication could occur in spite of antibody being present in the blood and tissue spaces. Indeed, in the unique situation found with virus tumors, it appears that the virus is present in each daughter cell following mitosis and, multiplying there, stimulates the daughter cell in turn to growth and division. Such a virus is capable of indefinite multiplication (as long as the tumor is growing) without ever being outside a cell.

The question of passive immunization is of interest in this connection. There is an analogy with certain of the bacterial toxemias, in that antibodies are unable to protect cells already parasitized but the presence of antibody may serve to prevent the further dissemination of infection. Practically, there is reason to think that virus may be widespread in a tissue and established in many cells before clinical signs of infection are apparent. The therapeutic effect of antiviral sera in general is not great.

Hypersensitivity.—A hypersensitive state has not been demonstrated in most of the virus diseases. In venereal lymphogranuloma, however, a skin reaction is produced in response to the intradermal inoculation of heated pus from infected bubos or of virus from other sources (p. 825). A skin reaction has also been produced in animals infected with the psittacosis virus.

Antigenic Analysis of Viruses.—With some of the smaller viruses, and those shown to consist of a single molecular species, it appears that a single antigen is responsible for the various serological reactions which may be demonstrated, such as neutralization, complement fixation and precipitation. Study of some of the more complex viruses has shown that soluble antigens demonstrable by complement fixation or precipitation technique may be separated from the virus itself. Indirect evidence of duality, at least, of antigens is obtained from a comparison of immunologic relationships among groups of viruses when tested by the neutralization test and by the complement fixation test. The pattern of relationship has been found to be quite different in some cases, depending upon which test is employed. For example see the psittacosis-lymphogranuloma group (p.819) and a neurotropic group (p. 844).

The most detailed examination of the antigenic make-up of a virus has taken place in Rivers' laboratory in a study of vaccinia virus.[31] A soluble antigen first described by Craigie in 1932 has been found to be protein in nature and to consist of two components (L and S) with different heat stabil-

[51] Craigie: Brit. Jour. Exp. Path., 1932, *13*:259.

ities. An "X" antigen, responsible for agglutination of elementary bodies, is a complex antigen, part of which may be nucleoprotein. In addition, there is an antigen of unknown nature responsible for the production of neutralizing antibody.

Non-Specific Factors in Resistance.—Age is a factor in resistance or susceptibility to many infectious agents, but several factors associated with age appear to be unique in influencing the pathogenesis of virus infections. Sabin[52] and his colleagues found that in young mice and guinea pigs one of several neurotropic viruses inoculated intramuscularly or by other peripheral routes, was able to progress to the central nervous system and produce infection there. As the animals became older certain barriers appeared so that virus no longer was able to reach the central nervous system after peripheral inoculation. The myoneural junction is apparently the site of one of these barriers, and others occur in the anterior rhinencephalon and the blood vessels. In this study age apparently did not influence susceptibility to intracerebral injection but it is known to do so in the case of rabies virus in mice and St. Louis encephalitis virus in rats.

When one considers that a virus is intimately dependent for its multiplication upon living host cells, it is not surprising to find that disturbances which affect the state of the cells or tissue may influence profoundly their susceptibility to virus infection. One of the most clear cut examples of this is found in the observation[53] that nerve cells are refractory to poliomyelitis virus for a time after section of their axons. A profound disturbance of the cell body occurs after such section, as evidenced by the reaction of chromatolysis and, although the refractiveness does not exactly parallel this visual evidence of an altered condition, the insusceptibility must depend upon what may be called a change in physiologic state of the cell.

Susceptibility of cells is also influenced by temperature. It is well known that fever, artificial or otherwise, will often bring on an attack of herpes (p. 806). Whether this is a direct effect of heat on the susceptible cells is unknown. Relatively small differences in temperature of experimental animals or tissue cultures sometimes determine whether growth of a virus will occur.

A number of investigations concerning the influence of *vitamins* upon resistance to virus infection have been reported. Thiamin deficiency in pigeons apparently activated a latent virus of the ornithosis group.[54] Vitamin deficiencies may retard the development of the natural barriers to virus migration described above. Observations with poliomyelitis virus suggested at first that vitamin deficiency rendered mice more resistant to infection, but it was later found that simple under-nutrition would produce the same result. This is more fully discussed on page 850.

Interference Phenomenon.—The presence of one virus in a susceptible tissue sometimes prevents the growth of a related strain in the same tissue. A number of examples of this phenomenon have been recorded and the evidence is strong that it does not depend upon the mechanisms usually

[52] Sabin and Olitsky: Proc. Soc. Exper. Biol. and Med., 1938, *38*:597.
[53] Howe and Bodian: Bull. Johns Hopkins Hosp., 1941, *69*:92.
[54] Pinkerton and Swank: Proc. Soc. Exp. Biol. and Med., 1940, *45*:704.

associated with the immune state. In 1935 Hoskins[55] reported that inoculation of a monkey with both virulent pantropic yellow fever virus and an avirulent neurotropic strain did not result in infection, provided the neurotropic strain was injected not later than twenty hours after the virulent strain was given. Similar observations have been reported for several other combinations of animal viruses and for plant viruses. Influenza virus[56] and bacteriophage[57] when treated with ultraviolet light to the point of destroying infectivity were still able to exert the interfering effect upon the same or related active strain.

The Nature of Viruses (Summary).—For many years the filterable viruses have been regarded as exceedingly small microorganisms differing only in size from bacteria. Proliferation and adaptive responses to altered environmental conditions are reasonably regarded as properties possessed by living as opposed to inanimate matter. There is, in fact, direct microscopical evidence that the elementary bodies of some viruses multiply by binary fission.

The obligate cytotropism of the viruses is perhaps to be regarded as evidence of a highly developed degree of parasitism approached by certain bacteria such as the leprosy bacillus, *Bartonella*, the spirochete of syphilis and others. The failure of the viruses to exhibit an independent metabolism has led many to believe that these agents are living microorganisms so degenerate that they are dependent upon the host cells for enzyme systems and are in the nature of "naked nuclei" or genes which adopt the protoplasm of the host as their own cytoplasm.[58]

In recent years the demonstration, by filtration experiments and other methods, of the exceedingly small size of some viruses, together with the isolation and study of the "virus proteins" of some of the plant viruses, has led many to question the animate nature of these agents. In the case of a very small virus, 10 to 15 $m\mu$ in diameter, there cannot be any great degree of organization, for there is room for only a few protein molecules. The "virus proteins" are regarded by many as autocatalytic substances lying in the borderland between the lifeless and the living.

Clearly, then, no conclusion can as yet be drawn regarding the general nature of the viruses. Two points should be noted in this connection. In the first place, the distinction between living and dead matter may perhaps become quite as pointless as regards the viruses as the once debated plant or animal nature of the bacteria. In the second, it is not only unnecessary but undesirable to assume that all viruses are of essentially the same nature; it is altogether possible, even probable, that some of the larger ones, such as the virus of vaccinia, are minute microorganisms which differ from bacteria only in size and cultural requirements, while very small viruses are homogeneous autocatalytic proteins.

[55] Hoskins: Am. Jour. Trop. Med., 1935, *15*:675.
[56] Ziegler and Horsfall: Jour. Exp. Med. 1944, *79*:361.
[57] Luria: Arch. Biochem., 1942, *1*:207.
[58] *Cf.* the discussion of Gortner: Science, 1938, *87*:529.

CHAPTER 37

VIRUS DISEASES OF MAN[1]

By F. B. Gordon, Ph.D., M.D.*

Virus infections of man produce many different clinical types of disease, and their heterogeneity in regard to this character as well as others exceeds that of the true bacterial diseases. Many of the most important infectious diseases of man are in this group, and although the technical difficulties of virus investigation have interfered with the accumulation of knowledge concerning some of these diseases, intensive investigation in other cases has uncovered sufficient facts to lead to valuable methods of control. The practical difficulties of propagation and identification of a virus, in contrast to a bacterium, are of particular significance in the laboratory diagnosis of the infections. In many cases there is no satisfactory method of laboratory diagnosis even in especially equipped laboratories, and the diagnosis is made by the clinician. There are a few exceptions to this at present, however, and with advance in knowledge the development of more satisfactory methods of laboratory diagnosis in this group of diseases may be expected.

In this chapter pertinent data concerning the virus diseases of man are brought together. Many of these viruses also produce spontaneous disease in animals. For information concerning virus diseases affecting only animals or plants the reader is referred to appropriate general references in Chapter 36.

SMALLPOX (VARIOLA) AND VACCINIA

Smallpox is a generalized disease characterized by skin lesions appearing first as papules and developing into vesicles and pustules which, after healing, may leave the deep, pitted scars that sometimes accompany recovery from this disease. Smallpox was recognized in China before the Christian era, and during the Middle Ages it was prevalent throughout Europe, reaching England and spreading to the New World in the sixteenth century. At the present time it is endemic in many parts of the world and epidemics are not infrequent, especially when prophylactic measures are relaxed.

Epidemiology.[2]—Smallpox is usually most prevalent in the winter or early spring months. It attacks all age groups but has appeared at times mainly as a disease of children, or at other times as a disease of adults,

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[1] Detailed information on certain aspects of many of these diseases may be found in *Virus and Rickettsial Diseases*. Harvard School of Public Health Symposium. Harvard University Press. Cambridge. 1940.

[2] *Cf.* Russell: *Epidemiology and Control of Variola*, pp. 176–200.[1]

depending upon the level of immunity, naturally or artificially acquired, in the population at the time.

The infectivity of a case is high and lasts from some time during the incubation period until well into convalescence. Virus is present in the skin lesions, remaining active for long periods in the dried crusts, and is no doubt also present in respiratory and buccal discharge, especially when lesions are present upon the mucous membrane of these regions. Transmission is by direct or indirect contact.

Observers have suspected for many years that smallpox exists in two forms—a severe "malignant smallpox," and a benign type which has been called by a variety of names (alastrim, cottonpox, kafirpox, paravariola, etc.). Convincing epidemiological evidence of the existence of two forms

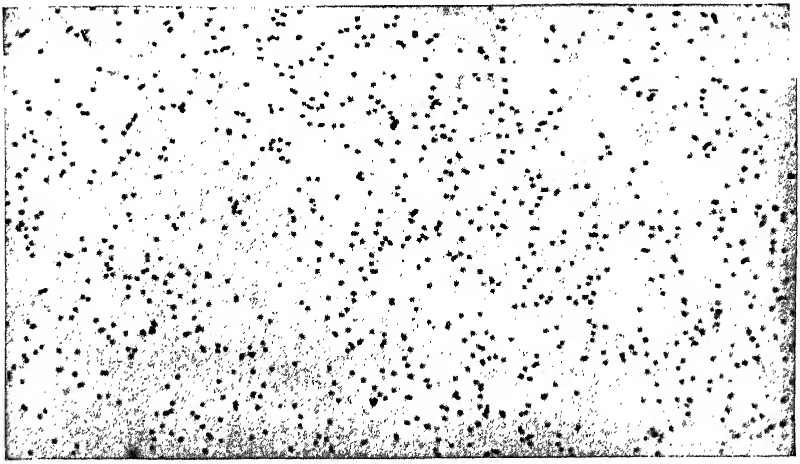

Fig. 228.—Elementary bodies of vaccinia; Morosow's stain; × 1000. From Parker and Rivers: Jour. Exp. Med., 1935, *62*:65. (Reproduced through the courtesy of the authors and publisher.)

of the disease has been presented by Chapin[3] and by Hedrick.[4] The case fatality in the mild form is less than 1 per cent; that in the malignant form 25 to 30 per cent. There is no evidence that there is a mutation of one strain of the virus to another. The mild form is the predominant type in this country at present, although the malignant form has occurred from time to time, apparently as the result of importation. Due to its mildness the former type is actually the more difficult to control, and there have been wide fluctuations in the number of cases reported in the Registration Area. In 1938 there were 14,939 cases (11.5 per 100,000) but in 1942 the figure was approximately 865 (0.6 per 100,000). The death rate has remained less than 0.1 per 100,000.

[3] Chapin: Jour. Prev. Med., 1932, *6*:273.
[4] Hedrick: Pub. Health Repts., 1936, *51*:363.

Vaccinia.—Some of the early investigators found that the agent of smallpox, when inoculated into calves or rabbits, was changed in nature and became the agent of vaccinia, used in immunization against smallpox. A preliminary passage through monkeys is said to facilitate this modification.[5] Vaccinia in man may be defined as a mild, usually local, disease transmitted by artificial dermal inoculation, and resulting in immunity against smallpox. Investigations of these two infections have gone hand in hand, and it is impracticable, if not undesirable, to treat them separately.

The Virus.—Although bacteria may often be found in variolous pustules, their irregularity of presence and type led the early observers to look further for an etiological agent. Attention was drawn to peculiar bodies within the epithelial cells of lesions of both variola and vaccinia, which Guarnieri in 1892 regarded as protozoa and named *Cytoryctes vacciniae et variolae*. These are now recognized as inclusion bodies (Guarnieri bodies). Paschen later described tiny corpuscular elements (elementary bodies) in vaccine lymph which are now regarded as particles of virus (p. 787 and Fig. 228). Numerous morphological studies have been made of Guarnieri bodies and vaccinal elementary bodies. Various observers have brought evidence that the Guarnieri body is made up of a colony of elementary bodies which are released when the cell and the contained Guarnieri body eventually are disrupted. This is the process depicted with another virus in Fig. 223. Morphological elements other than elementary bodies have been described in association with this virus. Disk-shaped forms and "spheres" similar to those seen in cultures of the pleuropneumonia agent have been observed.[6] The appearance of the elementary bodies under the electron microscope is shown in Fig. 226.

The virus of smallpox is said to be naturally infectious for monkeys as well as man, and if it is transmitted serially through monkeys it apparently keeps its identity, in contrast to the modification produced by passage in calves or rabbits. It also retains the character of variola virus when passed in fertile eggs, in which it has been propagated by several workers.[7] Vaccinia[8] is transmissible to many species of animals, but varying degrees of reaction are obtained. The rabbit is the common laboratory animal for the maintenance of this virus, and strains may be propagated in the skin, testis or brain. The inoculation of variolous material onto the cornea of a rabbit (Paul's test) has been used as a diagnostic procedure for smallpox. The development of a keratitis in which Guarnieri bodies are demonstrable microscopically indicates a positive test (Fig. 220).

[5] Attempts to produce the transformation are by no means always successful. For a review and account of a successful attempt see: Horgan: Jour. Hyg., 1938, *38*:702.

[6] Eisenberg-Merling: Jour. Path. and Bact., 1940, *50*:279.

[7] *Cf.* Nelson: Jour. Exp. Med., 1939, *70*:107.

[8] Because of the practical importance of the virus of vaccinia, and the ease of propagating large amounts of it in the laboratory, it has been intensively investigated and is regarded as a representative virus. To discuss the accumulated facts concerning it would require considerable space and much repetition of the material presented in the preceding chapter. For other specific information concerning the virus of vaccinia, therefore, the reader is referred to the appropriate section of Chapter 36, and to the review by Rivers in *Virus Diseases*. Cornell University Press. Ithaca. 1943, pp. 3–31.

Immunization against Smallpox.—Immunization against this disease, practiced from early times, was first accomplished by variolation, an artificial inoculation with smallpox virus. Crusts of smallpox lesions or the fluid from vesicles was applied in one of several ways to the skin or the nasal mucous membrane. The result was usually a mild case of smallpox with recovery, and the subject no doubt enjoyed a high degree of immunity against the natural disease.

The relation between human smallpox and cowpox, a disease manifested under natural conditions as a vesicular eruption on the udder, was clearly established in 1798 by the well-known observations of Jenner. Noting that dairy workers who had had cowpox were less liable than others to contract smallpox, Jenner advocated on this and other substantial grounds the systematic inoculation of cowpox virus as a protection against smallpox. The practical success achieved by this method of immunization was amply confirmed and is now a matter of common knowledge.

The Relation of Cowpox to Vaccinia.—The terms cowpox and vaccinia are usually used synonymously since the lesions produced by the two are quite similar grossly, and it has been shown repeatedly that strains of the two infections produce very good cross immunity. The ultimate origin of all strains of vaccine virus now used commercially is not known. It is assumed that some at least have been derived from variola, while others may represent the original cowpox strain used by Jenner.

Observations by Downie[9] have added evidence to previous suggestions that these two viruses are not identical. He has described differences in the histological appearance of the lesions and inclusion bodies of the two viruses when grown upon the chorioallantoic membrane of chick embryos and in animals. What is perhaps more significant, cross-serological tests (agglutination, complement-fixation, neutralization) with absorbed and unabsorbed sera showed that there exists a qualitative antigenic difference between the two types. Besides cowpox, there are a number of other "pock diseases" of animals which, it is thought, are related more or less closely to cowpox and variola. Immunological tests by the refined techniques now available would further an understanding of relationships within this group.

Vaccination.—Vaccine for human use is prepared by rubbing the virus into scratches on the shaved and carefully prepared abdomens of calves. The "seed virus" used for inoculation is kept fully active and at high titer by frequent passage through rabbits. After confluent vesicles have appeared along the scratches the virus is harvested by scraping off the vesicle walls and contents (vaccine "lymph") and emulsifying in glycerin. Bacterial counts are made, and cultural and animal tests are performed to detect *Cl. tetani* or other pathogens. After satisfactory tests for potency of the virus, the vaccine is tubed and stored at refrigerator temperatures.

Vaccination by the scratch method was popular at one time, but the method in favor at present is known as the "multiple-pressure" method,[10] which has the advantage of producing minimal scarring and being ap-

[9] Downie: Jour. Path. Bact., 1939, *48*:361.
[10] Leake: Pub. Health Repts., 1927, *42*:221.

parently less liable to bacterial infection. After the site is cleansed with acetone, a needle held horizontal to the surface is pressed repeatedly against the skin through a drop of vaccine, the elasticity of the skin causing the point of the needle each time to enter the epidermis. The excess vaccine is then wiped off and a dressing is not used.

A successful vaccination in a non-immune person results in the production after two or three days of a papule which develops into a vesicle, reaching its maximum on the eighth to twelfth day, and followed by a crust which finally drops off, leaving the characteristic scar. The "immune reaction,"[10] seen in immune persons, consists in the rapid development of a papule which begins to subside on the second or third day and never reaches vesicle stage. An "accelerated" or "vaccinoid reaction," probably indicating partial immunity, is similar to the true or "primary" reaction described above except that it is not so severe and runs a shorter and more rapid course.

In addition to calf lymph, tissue-culture virus,[11] virus grown in fertile eggs,[12] and filtered sheep-vaccine pulp[13] have been suggested and used to some extent for jennerian prophylaxis. They have the advantage of being bacteria free, which allows intradermal inoculation followed by immunity without the production of a scar.

Vaccination is recommended for infants during the first year of life. A first vaccination delayed until after the first year apparently increases the chances of "postvaccination encephalitis" (p. 814). A second vaccination during school age or later is regarded as providing a relatively safe level of immunity for life.

HERPES

Infection in man with the virus of herpes[14] is manifested by the appearance of a vesicular eruption on skin or mucous membrane. Often located at the borders of the lips or nares ("cold sores," *herpes labialis*), the vesicles are sometimes distributed bilaterally or may be more widespread. The mucosa of the genital tract may be attacked (*herpes genitalis*), and keratitis has been associated with this virus. Herpes often accompanies fevers of various types, particularly pneumonia and cerebrospinal meningitis ("fever blisters," *herpes febrilis*). It is not an infectious disease in the usual sense, but depends largely upon factors inherent in the patient. The slightest exposure to cold, a very mild nasal infection, emotional strain, or menstruation will incite a crop of herpetic eruptions in some individuals, while in others the stimulus must be more severe. Boak, Carpenter and Warren,[15] in work with artificial-fever therapy for syphilis and other infections, report that about 60 per cent of the patients suffer an attack of herpes simplex. Other observations[16] have shown rather conclusively that aphthous stomatitis, a common clinical condition in infants from one to three years of age,

[11] Rivers, Ward and Baird: Jour. Exp. Med., 1939, *69*:857.

[12] Buddingh: Amer. Jour. Pub. Health, 1937, *27*:1135.

[13] Henderson and McClean: Jour. Hyg., 1939, *39*:680.

[14] The disease described here is also called *herpes simplex* to distinguish it from *herpes zoster*, another disease which may also be of virus etiology.

[15] Boak, Carpenter and Warren: Jour. Bact., 1934, *27*:83.

[16] Dodd, Johnston and Buddingh: Jour. Pediat., 1938, *12*:95.

often accompanied by fever and toxic symptoms, is caused by the virus of herpes.

The possible relation between herpes and encephalitis lethargica is discussed elsewhere (p. 836), but it should be mentioned here that good evidence for the etiological rôle of herpes virus in a case of encephalitis in an infant has recently appeared. Smith and her associates[17] demonstrated typical herpetic inclusions in the brain tissue of the patient and recovered a strain of herpes virus by inoculation of mice.

The Virus.—Transmission was demonstrated in 1912 when Grüter showed that fluid from herpetic vesicles produced a keratitis when placed upon the scarified cornea of the rabbit. Herpetic lesions may also be produced in the rabbit's skin, and from both these sites virus may migrate along nerve pathways to the brain, producing an encephalitis.[18] Other animals exhibit varying degrees of susceptibility. A local vesicular lesion is produced after inoculation of the foot pad of a guinea pig, but the central nervous system is usually not invaded. An encephalitis follows intracere-

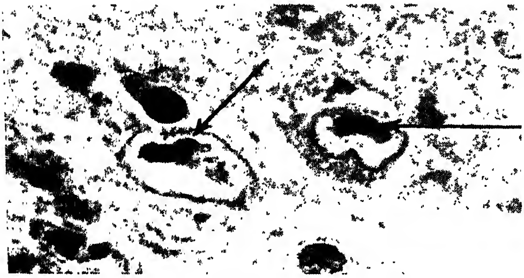

Fig. 229.—Intranuclear inclusion bodies of herpes. Two large nerve cells are seen, each containing an intranuclear inclusion. (From Smith, Lennette and Reames: Amer. Jour. Path., 1941, *17*:55. Reproduced through the kindness of the authors and pub lishers.)

bral inoculation of mice, and generalized infection of suckling mice has been described[19] following intranasal inoculation. The results of attempts to infect monkeys have been variable. Multiplication of the virus is easily obtained in tissue culture as well as upon the chorioallantoic membrane of the chick embryo.[20]

The characteristic intranuclear inclusion (type A), seen in infected tissues, is an acidophilic mass which almost completely replaces the nuclear chromatin. The latter accumulates at the nuclear membrane, a disturbance frequently called margination of the chromatin (Fig. 229; see also Fig. 222 for this type of inclusion). Some workers have reported the demonstration of elementary bodies in infected tissues, or within the inclusion body.[21]

[17] Smith, Lennette and Reames: Amer. Jour. Path., 1941, *17*:55.
[18] Goodpasture and Teague: Jour. Med. Res., 1923, *44*:139.
[19] Slavin and Berry: Jour. Exp. Med., 1943, *78*:321.
[20] Shaffer and Enders: Jour. Immunol., 1939, *37*:383.
[21] Nicolau and Kopciowska: Ann. Inst. Pasteur, 1938, *60*:401.

Herpes virus is relatively large, falling within the group having a diameter greater than 100 mμ. It may be stored for many months in infected tissue in 50 per cent glycerin, and some investigators have reported an enhancement of virulence by such treatment.

Immunity.—Although some local immunity may follow the skin lesions in man, auto-inoculation in another region produces infection, and apparently little or no general immunity occurs. Some persons are especially prone to herpes, and it has been postulated that the virus is latent at some focus within their bodies, manifesting its presence at intervals by the appearance of the eruption after fever or other suitable stimuli. Such a focus has not been satisfactorily demonstrated. The site of the skin eruption apparently does not harbor virus, because the healed lesion soon becomes non-infective. Burnet and Lush[22] considered the Gasserian ganglion to be a likely spot. The similarity to herpes zoster (p. 855), and the proved transmission of the virus along nerve tracts in animals, as well as observations suggesting a similar migration in man, indicate that the central connections of the fifth cranial nerve might harbor the virus. Inoculation of rabbits with the Gasserian ganglia taken at necropsy from six persons who had not had herpes with their last illness did not reveal the presence of the virus. Herpes virus has been demonstrated in saliva and spinal fluid of persons with and without herpes lesions, but the significance of these findings, in regard to carriage of the virus, is uncertain.

The results of neutralization tests with human serum show that most persons (60–90 per cent) possess antibodies against the virus of herpes. Several observers have noted a peculiar "all-or-none" result with human sera—either they have a high titer, or they are devoid of antibody. There is some evidence of a correlation between the presence of antibody and a tendency to recurrent herpes, suggesting that the antibody in man is the result of persisting latent infection.

In contrast to infection in man, herpes in animals is often followed by a general immunity, although sometimes of low grade. Immunity of the cornea of a rabbit following herpetic keratitis is said to last several months but finally to disappear. Immunity in the brain persists longer. Complement-fixing as well as neutralizing antibodies are present in the sera of hyperimmunized animals. Immune serum has been reported to protect mice and rabbits against the development of encephalitis after peripheral inoculation of virus, but this was not possible when virus was introduced into the brain.[23]

INFLUENZA[24]

Influenza occurred at least as early as the fourteenth century, and since the beginning of the sixteenth century waves of this disease have swept over the world at more or less frequent intervals, the latest being the great pandemic of 1918–19.[25]

[22] Burnet and Lush: Lancet, 1939, *i:629.*

[23] Hallauer: Ztschr. f. Hyg., 1937, *119:*505.

[24] See the review by Horsefall: *Virus Diseases.* Cornell University Press. Ithaca. 1943. pp. 113–143.

[25] For a review of the earlier literature, see Jordan: *Epidemic Influenza.* Amer. Med. Assn. Chicago. 1927.

Etiology.—The specific microorganism that was most commonly regarded as the cause of influenza following the pandemic in 1890 is the hemophilic bacillus discovered by Pfeiffer (*Hemophilus influenzae*, p. 460). This bacillus, although not found until 1892, after the initial wave of true influenza had passed, was generally regarded by bacteriologists up to 1918 as the causal agent of influenza. As a consequence of the bacterial observations made in 1918–20 and later, it is now established that *H. influenzae*, although often present as a secondary invader in influenza, is not the primary agent. A great variety of other microorganisms, such as streptococci, especially of the green-producing type, pneumococci, staphylococci, *Neisseria catarrhalis*, Friedländer's bacillus, and various nondescript microorganisms, are found similarly associated with cases of influenza both in

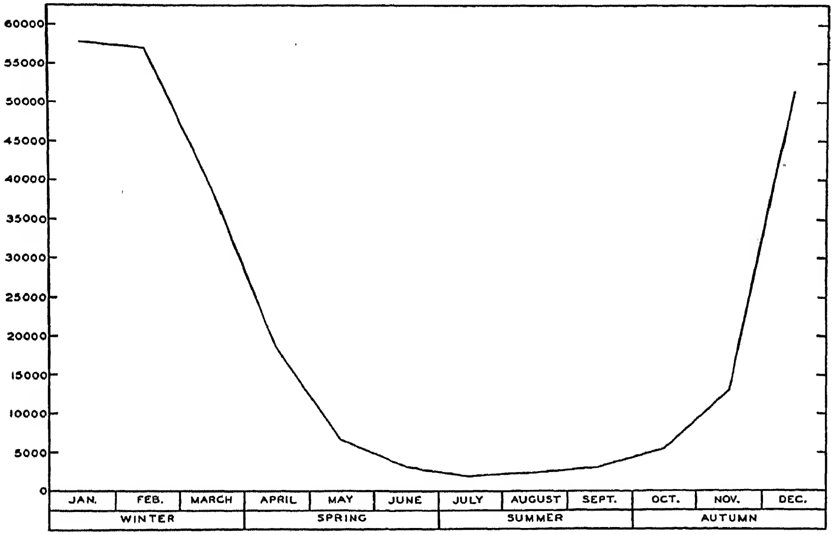

Fig. 230.—The seasonal incidence of influenza. Averages of reported cases by months for the years 1930 to 1938 inclusive. Data from Supplements to Public Health Reports.

the upper respiratory tract during the attack and in the pathological lesions after death.

The Virus.—Many of the features of influenza suggested that this disease is caused by a filterable virus, and since 1918 investigators have attempted to produce the disease through inoculation of filtered material. Many types of animals were used, but up until 1933 success was achieved only with chimpanzees.[26] The work of Smith, Andrewes and Laidlaw[27] (1933), who succeeded in establishing the disease in ferrets by intranasal inoculation with nasopharyngeal washings, marks the beginning of intensive and productive investigation of this disease. Influenza in ferrets re-

[26] Long, Bliss and Carpenter: Jour. Amer. Med. Assn., 1931, *97*:1112.
[27] Smith, Andrewes and Laidlaw: Lancet, 1933, *ii*:66.

sults in fever, inflammation of the nasal mucosa, and sometimes pneumonia, and transmission is accomplished by intranasal inoculation with filtered respiratory discharge or tissue emulsions. These results have been confirmed by others, and strains of influenza virus have been isolated in many parts of the world. These strains have all been isolated from "epidemic influenza;" the causative agents of the epidemiologically distinguishable "pandemic influenza" and "endemic influenza" remain unknown. It was found that ferret virus can be adapted to mice,[28] and other animals have shown varying degrees of susceptibility. Virus can be isolated and grown in the fertile egg and is recoverable in relatively high concentration and purity from the allantoic fluid.[29] Transmission of the animal strains back to man has been accomplished experimentally in the laboratory, thus completing the evidence of the etiologic role of this virus.

Influenza virus in throat washings may be preserved by mixture with equal parts of glycerin. Storage by freezing and drying or in the carbon dioxide refrigerator is easily accomplished. The virus may be inactivated by soaps, formalin and ultraviolet light without destroying the antigenicity. A detergent, "Zephiran," although virucidal in certain concentrations, may be diluted to a point where it will destroy the bacteria in throat washings but leave the virus active.[30] Propylene glycol vapor will destroy influenza virus suspended in the air.[31]

Early estimations of the size of the virus gave it a diameter of 80 to 120 mμ. More recent estimations based on sedimentation velocity and electron microscopy indicate sizes of 70 to 100 mμ, differing with the investigators and the strains of virus used.[32] Virus emulsions can serve as antigens in complement fixation tests with immune serums. The infective particle apparently acts as antigen but a "soluble" antigen, perhaps of protein nature, has also been demonstrated, especially in infected mouse lungs.[33]

In 1941 Hirst[34] reported that influenza virus, as contained in the allantoic fluid of infected eggs, has the ability to agglutinate chicken erythrocytes mixed with it. Although certain other viruses have been reported to act similarly, this observation (Hirst's phenomenon) has had its greatest application in investigations on influenza. The hemagglutinating power is closely related to infectivity, and the ability of antiserum to neutralize virus is reflected in its inhibition of agglutination of erythrocytes in the presence of virus. Thus, Hirst's observation has led to a valuable *in vitro* test which may be substituted for the more cumbersome neutralization test with mice.

Virus Strains.—Although it is certain that the strains isolated in ferrets are responsible for cases of influenza in man, in some epidemics it has been particularly difficult to isolate a virus, and human convalescents' sera have

[28] Francis and Magill: Science, 1935, *82*:353.

[29] Burnet: Austral. Jour. Exp. Biol. & Med. Sci., 1941, *19*:291.

[30] Personnel of U. S. Naval Lab. Res. Unit No. 1: Science, 1942, *96*:543.

[31] Henle and Zellat: Proc. Soc. Exp. Biol. Med., 1941, *48*:544; Robertson, *et al.*: Science, 1941, *94*:612.

[32] Stanley: Jour. Exp. Med., 1944, *79*:267.

[33] Lennette and Horsfall: Jour. Exp. Med., 1940, *72*:233.

[34] Hirst: Science, 1941, *94*:22.

in some cases shown no increased titer for the established ferret strains. During an epidemic of 1936–37 the British workers were able to recover strains of virus from as many as 75 per cent of cases by ferret inoculation. But in 1939[35] during early epidemics no strains were recovered from seventeen typical cases, and later only 12 per cent of cases yielded strains. This, together with the fact that only one-third of the convalescents showed an increase in serum-neutralizing titer, suggests that at least two etiological agents were present in 1939, one being difficult to isolate in ferrets and antigenically different from the known strains. No clinical differences were established between the cases which could or could not be classified as influenza on the basis of the serum tests or isolation of the virus. More recent findings have shed light upon this problem. In 1940 strains of influenza virus were isolated[36] which appeared similar in all respects to the strains previously studied except that they were completely different antigenically. Strains falling in this group are now called virus of influenza B and the earlier strains (*e.g.*, WS, PR8, Melbourne), as well as more recently isolated, antigenically similar ones, are known as virus of influenza A. Some cases of typical epidemic influenza yield neither of these viruses and fail to develop antibody against either, thus giving evidence that one or more types exist that have not yet been isolated.[37]

The virus strains of the influenza A group show some antigenic diversity demonstrable by cross immunity and cross neutralization tests in mice, but there is a broad general similarity. Smith and Andrewes[38] in a study of twenty-eight of these strains found that many antigenic components were present, but described four major antigens which served as a basis for a rough classification of the strains.[39] Ferrets and mice[40] immune to one strain of virus usually show varying degrees of immunity to others, although immunity to the homologous strain probably persists longer. Similar minor differences occur among strains of influenza B virus.

Immunity.—An attack of influenza produces only temporary immunity and repeated attacks in the same person have occurred. Most adults have antibody in their sera against influenza, demonstrable either by the neutralization or the complement fixation test, indicating previous contact with the virus. The level of antibody varies greatly from one individual to another, as might be expected, and an attack of influenza produces a transient rise in titer, which eventually returns to the previous level. This rise has been observed not only in frank cases but in individuals who have remained well during an epidemic. Although there is some correlation between amount of serum antibody and resistance to influenza,[41] it is apparent that other host factors play a rôle in determining whether a given dose of virus will result in infection. Attention has been focused on possible local factors in the respiratory tract. Abnormality of the

[35] Stuart-Harris, Smith and Andrewes: Lancet, 1940, *i*:205.
[36] Francis: Science, 1940, *92*:405; Magill: Proc. Soc. Exp. Biol. Med., 1940, *45*:162.
[37] Lennette, *et al.*: Pub. Health Rep., 1941, *56*:1777.
[38] Smith and Andrewes: Brit. Jour. Exp. Path., 1938, *19*:293.
[39] *Cf.* also Taylor, Dreguss, and Deritis: Amer. Jour. Hyg., Sec. B, 1940, *31*:36.
[40] Francis and Magill: Brit. Jour. Exp. Path., 1938, *19*:284.
[41] Rickard, *et al.*: Pub. Health Rep., 1941, *56*:1819.

nasal mucosa in the ferret, observed histologically, has been shown to influence susceptibility in that animal. The significance of a virus neutralizing substance found in human nasal secretions,[42] and at least related to serum antibody, is not yet known.

Active Immunization.—Parenteral inoculation of experimental animals with influenza virus will protect them against a subsequent intranasal inoculation. This can be accomplished with virus rendered non-infective in a number of ways as well as with active virus. Similar inoculation of man with various virus preparations induces an increased serum antibody titer, and susceptibility to subsequent induced infection can be lowered by such procedures. Many of the attempts to protect man against natural exposure by active immunization have given negative or equivocal results, no doubt in part due to the diverse etiology of human influenza, but other factors may also be of significance. However, a preliminary report[43] of the results of an extensive field trial indicates that a polyvalent vaccine (containing both A and B strains) lowered the incidence of clinical influenza during a subsequent epidemic period in all the groups to which it was given. The vaccine was made from concentrated virus in allantoic fluid, rendered non-infective by the addition of formaldehyde in 1:5000 dilution, and given subcutaneously in a single dose of 1 cc.

Passive Immunization.—Injection of immune serum has a protective effect against intranasal inoculations of the virus in mice and the effect is much greater if the serum is given intranasally. Favorable results have been reported[44] in an attempt to protect man during an epidemic of influenza by allowing the subjects to inhale finely dispersed droplets of immune horse serum. Inhalation of immune human serum was found in other experiments[44a] not to protect human volunteers against artificial infection induced by inhalation of finely dispersed virus suspension.

Swine Influenza.[45]—A respiratory disease of swine in the Midwest was observed at the time of the 1918 pandemic of influenza in man, when its similarity to the human disease was noted. The elucidation of its etiology, antedating that of human influenza, appeared in 1930, when Shope found that two agents are associated with the disease, both of which must be present to produce the typical clinical syndrome. He isolated a filterable virus and a hemophilic organism very similar to Pfeiffer's bacillus, which he called *Hemophilus influenzae suis*. The virus alone when inoculated into a pig produced a minimal, sometimes inapparent, infection termed "filtrate disease." The simultaneous inoculation of the hemophilic organism or the inoculation of the virus into a pig which was a carrier of this bacillus resulted in production of typical swine influenza. These findings suggested that the disease in man was of a similar dual nature, but subsequent investigation with human strains has shown that the virus itself is capable of producing the complete disease picture.

[42] Burnet, Lush and Jackson: Brit. Jour. Exper. Pathol., 1939, *20*:377.
[43] Commission on Influenza: Jour. Amer. Med. Assn., 1944, *124*:982.
[44] Smorodintseff, Gulamoff and Tschalkina: Zeitschr. f. Klin. Med., 1940, *138*:756.
[44a] Report of Commission on Influenza, U. S. Army, April, 1944.
[45] *Cf.* Shope: *Virus Diseases.* Cornell University Press. Ithaca. 1943. pp. 85–109.

Swine influenza appears on midwestern farms in the autumn and spreads with great rapidity. Shope, while not denying the contagious nature of the disease, questions whether the rapid spread can be accounted for on the basis of contact, and suggests that the sudden appearance of the disease in a previously healthy herd may be due to some "exciting" cause acting in a herd in which carriers of the virus are already present. He has discovered a complex cycle[46] of transmission, involving two reservoir hosts, which represents a possible means of dissemination of the virus in a masked form throughout a herd. He has shown that lungworms (nematodes) from infected swine contain the virus. The lungworm ova are excreted in the pig's feces where they are ingested by earthworms in which the larvae appear. The cycle is completed when the earthworm is eaten by a pig, in which the adult worms develop. Virus is carried by larvae developing from ova laid by worms in an infected pig, and reaches another pig when the infected larvae within the earthworm are ingested. The development of the clinical disease, however, does not occur until some exciting cause comes into action. Artificially, an injection of *H. influenzae suis* can act as the exciting cause but climatic conditions also may be concerned. Natural epizootics of swine influenza occur only during the winter months (October to April) and it is only during this period that experimental transmission by lungworms is demonstrable.

The virus of swine influenza is closely related to the human strains, both ferrets and mice being susceptible to swine as well as human strains, and both types of strains producing similar infections in swine. Cross-immunization tests indicate that there is some antigenic overlapping between human influenza A and swine strains.

PRIMARY ATYPICAL PNEUMONIA

During the years 1940 to 1942 an influenza-like disease of the respiratory tract gained general recognition, although the first observations had been recorded some years earlier. This clinical entity was first described under a variety of names but the officially suggested term "primary atypical pneumonia, etiology unknown"[47] soon came into general use.

Epidemic and sporadic cases are recognized, the former having been observed especially among army and navy personnel, among which the disease has been a potentially serious cause of incapacitation. The sporadic cases in general have been more severe and presented a wide variety of clinical characters, while the epidemic form is usually milder, many cases exhibiting only an upper respiratory infection. It should be borne in mind, however, that mild cases are much more readily recognized during an epidemic and we have no real evidence that the infectious agents found in the sporadic type may not also produce mild infections, the true nature of which goes unrecognized. One of the characteristics of this disease, recorded by many observers, is the relatively severe pulmonary involvement, revealed by the x-ray, which may be present when the physical signs and general condition of the patient give no hint that the lower respiratory tract is affected.

[46] Shope: Jour. Exp. Med., 1941, *74*:49.

[47] War Medicine, 1942, *2*:330.

No obvious bacterial etiologic agent has been discovered in this disease and, in contrast to the usual pneumonias, these cases are not modified by treatment with the sulfonamides. A number of non-bacterial agents have been shown to be related etiologically to sporadic cases falling within the clinical definition of primary atypical pneumonia. Thus, several of the viruses of the psittacosis-lymphogranuloma group (p. 819) produce this type of illness. Lymphocytic choriomeningitis virus (p. 827) may produce an influenza-like disease, and infection with *Rickettsia diaporica* (p. 776) has on occasion taken a pulmonary form. Primary coccidioidomycosis (p. 665) also falls within the clinical description of this disease. In the epidemic cases, however, failure to isolate the agents enumerated, as well as negative serological findings, indicates that most of these cases, at least, have a different causative agent.

No less than six viral agents, other than influenza virus and those mentioned above, have been isolated by various investigators during a search for the cause of primary atypical pneumonia. Varying amounts of evidence have been submitted for the etiologic significance of each but further observations are necessary before a final evaluation can be made. References to these investigations may be found in the report by Eaton and his colleagues who have attacked this problem with some success. They describe the isolation of a virus in cotton rats by inoculation of the sputum from patients. This agent is also infective for hamsters and can be propagated in the egg by inoculation into the amniotic cavity.[48] The evidence is good that it is the cause of some cases, at least, of this clinical syndrome.

Another group of investigators[49] offer evidence that an "indifferent" streptococcus plays some role in this disease.

"Cold" Hemagglutinin.—An interesting observation was made by Peterson, Ham and Finland,[50] and confirmed by others, that serum from cases of primary atypical pneumonia will agglutinate human erythrocytes in the cold. The reaction is demonstrable at 4° C. but not at 37° C., the agglutination being reversible as the tubes are warmed. The autohemagglutinin titer of the patient's serum begins to rise during the second week of illness and persists for a month or more. This reaction, although described for a number of other conditions also, is of value in diagnosis. It is apparently negative in those cases due to viruses of the psittacosis group.

THE COMMON COLD

One of the chief causes of disability the world over is the acute affection of the upper respiratory tract known as the common cold. The symptoms are diverse and need little description since they are experienced by most persons two or three times a year. The "sickness surveys" usually list more persons temporarily incapacitated from this cause than from any other.

Etiology.—It is not necessary to assume that all colds, although they may be very similar in their character and sequelae, are caused by the same agent. The initial symptoms of measles and a number of other dis-

[48] Eaton, Meiklejohn and Van Herick: Jour. Exp. Med., 1944, *79*:649.
[49] Thomas, *et al.:* Science, 1943, *98*:566.
[50] Peterson, Ham and Finland: Science, 1943, *97*:167.

eases are very similar to those of the common cold; experimental spraying of the upper respiratory tract with pure cultures of the Pfeiffer bacillus and of some other bacteria has, in several instances, produced symptoms of the common cold. At the same time painstaking examination of the bacterial flora of the mouth and nose extending over considerable periods has shown that there is no significant increase in number or change in type of bacterial flora coincident with the development of a cold in the individual under observation. Furthermore, inoculation of volunteers with strains of a variety of organisms isolated from patients with colds has given no evidence of a consistent causal relationship. Many investigators have hence been driven to search for a more specific cause which shall explain, among other things, the great communicability of colds. This has been found, apparently, in a filterable virus, which gives rise to colds in human volunteers and in chimpanzees.

The Virus.—Observations by Kruse[51] in 1914, by Foster[52] in 1916, and later by others, gave indications of the presence of a filterable virus in colds, but several subsequent investigators failed to get similar results. More convincing evidence in favor of the virus origin of colds has been forthcoming in the work of Dochez[53] and his associates in New York City and in similar investigations at Johns Hopkins University.[54] It has been shown that chimpanzees are susceptible to colds which are practically identical with colds observed in man; that chimpanzees and human beings given intranasal instillations of bacteria-free filtrates of nasal washings from early human cases of colds develop an acute respiratory infection indistinguishable from the natural common cold; that this disease can be transmitted through a series of human volunteers or experimental animals by successive inoculations of bacteria-free filtrates from nasal washings. No other infectious agent has yet been shown to bear any such constant and definite relation to any type of the common cold.

Dochez and his associates found that the virus of the common cold is cultivable upon chick-embryo medium but prolonged passage leads to a loss of activity. The virus may be preserved by freezing and drying infective nasopharyngeal washings or tissue culture, and in nasal washings it will survive at least thirteen days under anaerobic conditions in the refrigerator.

Few beliefs are more firmly fixed in the popular mind than that atmospheric changes, especially sudden drops in temperature, are responsible for outbreaks of colds. Two investigators[55] who spent eleven months studying the epidemiology and bacteriology of colds in Spitzbergen, an isolated arctic community, cut off completely from the outside world for several months each year, noted that the arrival of the first boat of the shipping season was followed by a sudden epidemic of colds which involved the whole community in a short period of time. Similarly Robert Marshall[56]

[51] Kruse: Münch. med. Wchnschr., 1914, *61*:1547.

[52] Foster: Jour. Amer. Med. Assn., 1916, *66*:1180; Jour. Inf. Dis., 1917, *21*:451.

[53] Dochez, Mills and Kneeland: Jour. Exp. Med., 1936, *63*:559; Dochez: Jour. Amer. Med. Assn., 1938, *110*:177.

[54] Long, Doull, Bourn and McComb: Jour. Exp. Med., 1931, *53*:447.

[55] Paul and Freese: Amer. Jour. Hyg., 1933, *17*:517.

[56] Marshall: *Arctic Village*. Harrison Smith & Robert K. Haas. New York. 1933.

notes that the visits of the mailcarrier from the Yukon to the upper Koyukuk are often followed by a general outbreak of colds among the Koyukuk residents. It is evident that changes in weather conditions, whatever effect they may have in promoting an increased susceptibility, are not the necessary primary exciting cause of an epidemic of colds. Certain puzzling epidemiological factors in the dissemination of colds "without apparent contact" may be explained by an observation on an experimental chimpanzee colony[57] which suggests that colds may be infectious during the incubation period before the onset of any clinical symptoms or signs of the disease.

Prophylaxis.—Control of the common cold is an even more difficult task than that of most droplet-borne infections because of the well-nigh universal distribution of the virus. Favorably situated individuals may perhaps do something to minimize contacts, but the average active person in an urban community is likely to be rather frequently exposed. Many measures of personal hygiene have been advocated to lower susceptibility. Attempts to control the diet by various means, such as giving large doses of vitamins A and D, furnish no evidence that children so treated have a lower incidence of respiratory diseases than others. Exposure to ultraviolet rays does not diminish the frequency of the common cold in those treated.[58] Various "hardening" procedures such as sleeping in rooms with wide open windows or taking systematic outdoor exercise do not seem to be noticeably efficacious in preventing colds.[59]

Recovery from a cold apparently is accompanied by a transient immunity, if any; some persons can experience several attacks within a period of a few months. Attempts to immunize man with active virus have been unsuccessful, but various "cold vaccines," consisting of killed bacterial emulsions, are on the market. Their efficacy in preventing colds is questionable, although they have considerable psychological effect. Some may be of value in preventing infection by secondary invaders, following primary infection of the cold virus.

MEASLES

Although measles is one of the commonest diseases of childhood, it readily attacks adults in populations not previously exposed, or in groups in which an appreciable number of susceptibles are present. During World War I it was prevalent in the Army camps of the United States but it has not been a problem among the troops mobilized since 1940. There is a tendency to look upon measles as one of the harmless diseases of childhood because of its general prevalence. Although it has a low case fatality rate, over 1200 deaths were recorded in the Registration Area during 1942. The reported cases were more than one half million, a rate of 409.7 per 100,000 persons.

The disease is characterized in the prodromal stage by an upper respiratory infection with conjunctivitis, catarrh and fever. Later the characteristic enanthem in the buccal mucosa and the exanthem of the skin appear.

[57] Long, Bliss and Carpenter: Bull. Johns Hopkins Hosp., 1932, *51*:278.
[58] Doull, Hardy, Clark and Herman: Amer. Jour. Hyg., 1931, *13*:460.
[59] Gafafer: Amer. Jour. Hyg., 1932, *16*:233.

Although not dangerous when uncomplicated, measles may be followed by streptococcal or pneumococcal pneumonia or other secondary infections. The virus is present in nasopharyngeal secretions and a case is infectious in the pre-eruptive stage. Infectivity is high and almost all exposed children develop the disease.

The Virus.[60] Green-producing streptococci have been isolated by several investigators[61] from blood and secretion of measles cases, but they are not generally accepted as the primary agent and their significance is questionable.

Hektoen[62] found that blood drawn from the veins of a patient and mixed with ascitic broth gave no visible growth when incubated at 37° C. for twenty-four hours. But a few cubic centimeters of this mixture injected into a healthy man produced the typical symptoms and eruption after the usual period of incubation. Sellards,[63] however, in carefully controlled observations upon eight subjects failed to transmit measles by the injection of blood.

Anderson and Goldberger[64] seemingly succeeded in communicating the disease to the lower animals (monkeys) in definite and regular fashion. The apparent reason for the preponderating negative and irregular results of earlier experimenters is the fact that human blood is infective for monkeys during a very limited period. This period begins before the appearance of the characteristic eruption and continues only a limited time thereafter.

Further experiments by the same investigators[65] demonstrated the presence of the virus of measles in the mixed buccal and nasal secretions, and showed that the virus could pass through a Berkefeld filter, could resist desiccation for twenty-five and a half hours and freezing for twenty-five hours, while infectivity of the virus was destroyed by heating for fifteen minutes at 55° C.

Subsequently other investigators confirmed the susceptibility of monkeys and the presence of virus in blood and throat washings.

Several attempts have been made to culture the virus on fertile eggs. Rake and Schaffer[66] reported the passage of the virus through more than twenty serial egg passages, starting with blood or nasopharyngeal washings. The agent apparently multiplies on the chorioallantoic membrane but produces no constant lesion to denote its presence. Inoculation of monkeys with the culture virus is followed by one or more of the signs of measles in man—fever, coryza, leucopenia, enanthem and exanthem.

During the course of the egg cultures these investigators found further evidence that the agent belongs to the group of filterable viruses. It passed through a Seitz EK filter, and withstood treatment with ether sufficient to eliminate bacterial contamination. It can also be stored at the temperature of dry ice.

[60] See the survey of recent work by Enders: *Etiology of Measles*, pp. 237–267.[1]
[61] *Cf.* Tunnicliff: Jour. Amer. Med. Assn., 1930, *95*:4.
[62] Hektoen: Jour. Inf. Dis., 1905, *2*:238.
[63] Sellards: Bull. Johns Hopkins Hosp., 1919, *30*:257.
[64] Anderson and Goldberger: Pub. Health Repts., 1911, *26*:847, 887.
[65] Goldberger and Anderson: Jour. Amer. Med. Assn., 1911, *57*:476, 971.
[66] Rake and Schaffer: Jour. Immunol., 1940, *38*:177.

Immunity.—One attack of measles confers immunity; second attacks are very rare. The serum of convalescents and "normal" adults, most of whom have had measles, is of value in prophylaxis. Doses of 5 to 10 cc. given after exposure but before the onset afford protection. The *desideratum* is to allow a mild infection to occur, sufficient to give the subject an active immunity. A recent report[67] indicates that larger amounts of serum given as late as the catarrhal stage are able to modify the severity of the attack.

Rake and his colleagues found that the measles virus, after passage in eggs, gave a much modified, mild disease in children. Inoculation with the egg-passage virus apparently conferred appreciable resistance against the disease, whether the exposure was by chance contact with natural cases or by actual inoculation with blood from known cases.

MUMPS

This highly contagious disease of man, often contracted in childhood, usually takes the form of a swelling of the parotid gland, and is sometimes called *epidemic parotitis.* This gland is not the only vulnerable organ, however; the submaxillary and sublingual glands, testis, ovary, pancreas, and perhaps other glands are attacked, either accompanying or in the absence of parotid involvement. An unknown fraction of patients also exhibit signs of encephalic disturbance, which may be very mild and manifested only by pleocytosis of the spinal fluid, or may be more severe. Inflammation and atrophy of various cranial nerves have also been seen associated with mumps. As in the case of measles, convalescent human serum may be used for prophylaxis after known exposure and during the incubation period, which averages about eighteen days.

A number of experimenters have produced pathological changes in animals by inoculation with material from cases of mumps, but because of inability to pass the infection indefinitely in animals, or for other reasons, most of these reports do not convince the reader that the etiological agent of mumps was demonstrated. Johnson and Goodpasture,[68] however, have submitted convincing evidence that an agent present in the saliva of early cases of mumps, when inoculated into Stensen's duct of monkeys, causes experimental mumps. The agent is filterable, resists freezing and drying, may be preserved in glycerin, and was carried through fourteen transfers in monkeys. The monkey passage virus was sprayed into the mouth and nose of 13 supposedly susceptible and 4 immune persons, with the result that 6 of the former group showed typical, and 3 questionable, symptoms, while the 4 immunes showed no evidence of infection. Findlay and Clarke[69] have confirmed the isolation of virus in monkeys and found that it will produce an orchitis in these animals.

Enders and Cohen have described a complement-fixation test using infected parotid gland of the monkey as the source of antigen. Positive tests correlated well with a history of mumps but about 50 per cent of individuals

[67] Maris, *et al.:* Jour. Pediat., 1943, *22:*17.

[68] Johnson and Goodpasture: Jour. Exp. Med., 1934, *59:*1; Amer. Jour. Hyg., 1935, *21:*46.

[69] Findlay and Clarke: Brit. Jour. Exp. Path., 1934, *15:*309.

without a history of mumps showed serum antibodies, suggesting that these persons had undergone an unrecognized infection. A skin test for detection of resistance or susceptibility was also used in this study.[70]

THE PSITTACOSIS-LYMPHOGRANULOMA GROUP

It has recently been recognized that a number of viruses, whose relationship was not formerly suspected, are in fact similar, and constitute a more or less definite group[71] on the basis of morphologic, antigenic and certain other relationships. Included within this group are several animal strains as well as the following viruses affecting man; psittacosis, ornithosis, certain strains from human pneumonitis, lymphogranuloma venereum, and less definitely related, trachoma and inclusion conjunctivitis.

These agents all have in common the presence of a characteristic coccoid elementary body demonstrable in smears of infected tissues (Fig. 231), and all the evidence indicates that these are the infective particles of the virus. They may be stained by Giemsa, Castaneda's rickettsial stain, or by Machiavello's method.* In the case of several of these viruses the elementary bodies have been shown to be one stage in a developmental cycle which is described (p. 822) in the discussion of psittacosis virus.

All the members of this group are infective for mice by the intranasal route, producing a pneumonitis, and many are also pathogenic by the intracerebral or intraperitoneal route. They have some morphological resemblance to the rickettsiae and, like these agents, multiply to a high titer in the yolk sac of fertile eggs. Antigenic similarities have been described between various members of the group on the basis of the complement-fixation test,[72] a test which apparently depends upon an antigen distributed through the group. In contrast, Hilleman[73] has reported quite sharp antigenic differences on the basis of neutralization tests.

Psittacosis.[74] This disease, known during the past sixty-five years, was brought into special prominence by the large number of cases appearing in Argentina, Europe and North America[75] in 1929–30. The disease appears in persons associated with birds, and manifests itself in the form of small outbreaks confined to members of families or to people living or

* The last named, because of the marked contrast between color of elementary bodies and tissue elements, is the most satisfactory for general purposes. The method is as follows:

a. Flood the dried slide with 0.25 per cent basic fuchsin freshly made up in phosphate buffer of *p*H 7.4. Leave for five minutes.
b. Destain five to ten seconds with 1 per cent citric acid and wash quickly with tap water.
c. Stain ten seconds with 1 per cent aqueous methylene blue.

The elementary bodies stain with carbol fuchsin and retain the color in the presence of weak acid. Tissue elements are destained by this method except in the thicker portions of the smear. Destaining should progress only to the point where the thinner portions of the smear are pinkish-grey in color.

[70] Enders: Ann. Intern. Med., 1943, *18:*1015.
[71] Beck, Eaton and O'Donnell: Jour. Exp. Med., 1944, *79:*65.
[72] Eaton and Corey: Proc. Soc. Exp. Biol. and Med., 1942, *51:*165.
[73] Hilleman: Jour. Inf. Dis., 1945, *76:*96.
[74] *Cf.* Enders: *Psittacosis*, pp. 528–555.[1]
[75] Armstrong: Pub. Health Repts., 1930, *45:*2013.

working in the same location. The connection with birds is always striking and transmission to man seems to be almost invariably from infected birds. Occasional instances of apparent transmission from one human being to another have, however, been observed.

Although a number of species of birds are susceptible, infection of man has been brought about almost entirely by contact with parrots or parakeets, including Australian budgerigars. Meyer, recognizing that other than psittacine birds are affected with this type of virus and can transmit the infection to man, has proposed the more inclusive term "ornithosis."[76] At the present time both terms are in use, psittacosis retaining its former connotation and ornithosis (p. 823) indicating infection with similar viruses from non-psittacine birds.

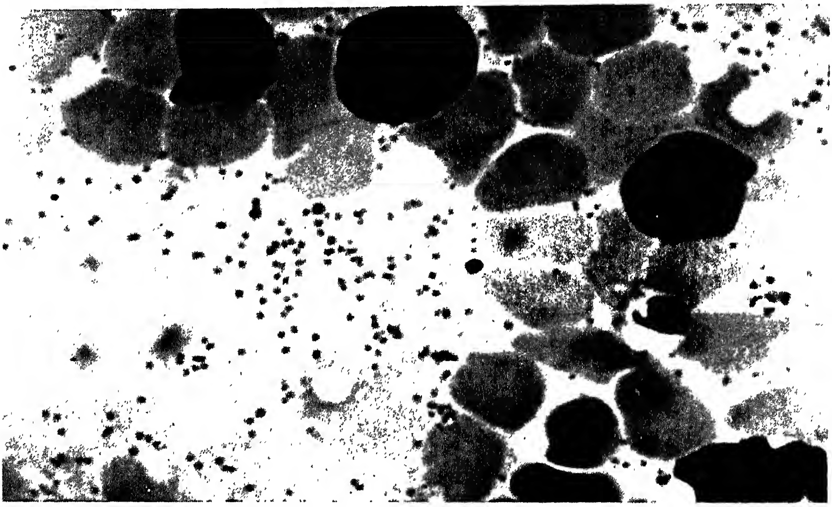

Fig. 231.—Elementary bodies of psittacosis. Impression preparation from infected mouse spleen; reduced from × 2575. (Obtained through the courtesy of S. P. Bedson.)

The Avian Disease.—Infection with the virus of psittacosis appears to be enzootic among psittacine birds in the natural state, at least in Australia, and the disease has been discovered in birds imported from several countries. Birds in the acute stage of the infection show emaciation, weakness and ruffling of the feathers. A nasal discharge and the watery cloacal excretion both may contain the virus. The conspicuous pathologic lesion is a focal necrosis of the liver. The incubation period may be very long and recovery may result in a prolonged carrier stage. Healthy-appearing carriers of the virus make it difficult to prevent importation of infected stock.

The Human Disease.—It is assumed that the portal of entry in man is the respiratory tract. Contact with infected birds is unnecessary, indirect

[76] Meyer: Medicine, 1942, *21*:175.

contact through fomites, or even proximity to infected material, being sufficient to cause infection in man. The degree of infectivity, approaching that of measles and smallpox, as indicated in a study by McCoy, has made laboratory investigation of this disease particularly difficult.[77] The infection in man is often severe, and a rather high case-fatality rate (20–30 per cent) is the rule. It usually has an acute onset and takes the form of a pneumonia, with, however, a paucity of sputum (*cf.* Primary atypical pneumonia, p. 814).

A method of active immunization, first proved of value in experiments with monkeys, has been used for protection of laboratory workers against this disease.[78] Active virus inoculated intramuscularly, apparently without danger, results in the appearance of neutralizing antibodies in the serum. Tests in monkeys indicate that an increased resistance to virus introduced into the respiratory tract is also present.

The Virus. For some time after 1893, when Nocard isolated a gram-negative bacterium from the bone marrow of parrots which had died with psittacosis, it was thought that the causative agent had been found. Subsequent work showed that this organism, which turned out to be *Bacterium typhi-murium*, was not of etiological significance. Bedson, Western and Simpson in 1930[79] first demonstrated that the disease is caused by a filterable virus, and this has been amply confirmed by others.

The virus is present in the blood and sputum of patients with the disease and in the tissues of infected animals. After intraperitoneal inoculation of a mouse, the animal used mainly for investigation of this disease, virus is present in the spleen, and intranasal inoculation produces a pneumonitis. The virus has been preserved by removing mouse spleens and storing in the refrigerator in phosphate buffer at *p*H 7.6. Activity is not well preserved in 50 per cent glycerin. Intradermal injection of virus into the skin of rabbits and guinea pigs produces a papular eruption similar to that caused by herpes virus. A pneumonia not unlike that seen in man follows intratracheal inoculation of monkeys, and a meningo-encephalitis develops after intracerebral injection of mice, monkeys, rabbits and guinea pigs.[80] Cultivation in tissue culture and on the chorioallantoic membrane of eggs is successful,[81] but a more susceptible tissue is the yolk sac.

Examination of infected material shows the presence of small coccoid bodies, arranged singly and in pairs, illustrated in Fig. 231. They can be stained by the methods indicated on p. 819. Centrifugal sedimentation and serological reactions furnish evidence that these bodies are the virus. Levinthal first called attention to considerable variation in size and form of these structures and gave them the name *Microbacterium multiforme psittacosis*. Lillie proposed the name *Rickettsia psittaci*, but this has not been accepted because no arthropod host has been demonstrated. They

[77] McCoy: Pub. Health Repts., 1930, *45*:843.

[78] Rivers and Schwentker: Jour. Exp. Med., 1934, *60*:211.

[79] Bedson, Western and Simpson: Lancet, 1930, *i*:235, 345.

[80] Rivers, Berry and Sprunt: Jour. Exp. Med., 1931, *54*:91; Rivers and Berry: *ibid.*, 105, 119, 129.

[81] MacCallum: Brit. Jour. Exp. Path., 1936, *17*:472: Burnet and Rountree: Jour. Path. Bact., 1935, *40*:471.

are often called elementary bodies of psittacosis and, because they were described at about the same time by the two workers mentioned above and by Coles, they are also known as L.C.L. bodies.

Developmental Cycle.—A number of investigators have studied the morphology of this virus and have found that the elementary bodies represent only one stage in a developmental cycle. The descriptions by various observers,[82] although differing in minor respects, are in general agreement and may be summarized as follows:

The elementary body, estimated at 200 to 300 mμ in diameter, upon entering a susceptible cell increases in size to become an "initial body" with a diameter two to four times that of an elementary body. With multiplication by fission (according to most observers) and the development of a matrix, which may be produced by the virus or by the host cell, a "plaque" is formed which may be several microns in diameter. With

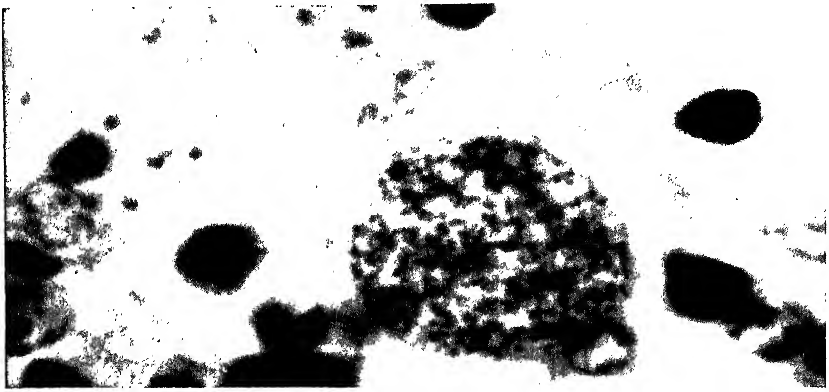

Fig. 232.—An intracellular vesicle containing elementary bodies from the lung of a mouse infected with mouse pneumonitis. This is similar to the final stage in the developmental cycle of psittacosis virus. Noble's stain (see ref. 82); × 2100.

many stains this appears to be a homogeneous structure, but proper manipulations reveal corpuscular elements in the process of division within the matrix. As development proceeds the matrix material becomes less dense, revealing particles which become of smaller average size during continued division until eventually a large intracellular vesicle (Fig. 232) is formed consisting of a mass of elementary bodies. This vesicle eventually ruptures, releasing elementary bodies for the infection of other cells. The whole cycle requires forty-eight to seventy-two hours. An interesting observation recorded by two different observers (and also described as occurring in the similar cycle of lymphogranuloma venereum virus) is that the sequence is different if the infective particle enters a cell already damaged. In this case no plaque is formed; the elementary bodies multiply

[82] See the summary of previous work and observations recorded by Yanamura and Meyer: Jour. Infec. Dis., 1941, *68*:1.

by continued fission retaining the general size and character of elementary bodies until the vesicle stage is reached.

The similarity between viruses of this group and the rickettsias is evident also in a comparison of these developmental forms with certain morphologic elements of the rickettsias observed in infected animal lungs (p. 768).

Laboratory Diagnosis.—Rivers and Berry[83] have described a rapid diagnostic method for the human disease which consists of inoculating mice intraperitoneally with filtered or unfiltered sputum. Specific infection in the mice may be recognized by typical lesions and the presence of L.C.L. bodies in spleen and liver, or by subsequent immunity to inoculation with a known strain of psittacosis virus. Complement-fixation tests, also of value in the diagnosis of human infections, have been found positive as early as the twelfth day of the disease. This test has been of value also in the recognition of latent infection in psittacines imported into this country.

Ornithosis.—A number of strains of psittacosis-like virus have been isolated from birds other than parrots and parakeets, and the term ornithosis has come to be used to distinguish these from true psittacosis strains. A disease occurring in the Faroe Islands and traced to contact with fulmar petrels yielded in 1938 a virus similar to psittacosis, and this was one of the first indications that viruses of this type occurred in other species than those of the *Psittacidae*. On numerous occasions pigeons have been shown to be infected, and atypical pneumonia in man has been traced to contact with pigeons. Domestic fowl also can harbor ornithosis virus and presumably transmit the infection to man. Various domestic birds of Michigan have yielded sera capable of fixing complement with meningopneumonitis antigen, thereby indicating infection with a virus of this group. Similar strains have been isolated from cases of atypical pneumonia in man.

Representative strains of ornithosis virus differ slightly from psittacosis strains in pathogenicity for experimental animals, and Hilleman[73] has found that they are antigenically quite different as determined by a neutralization test.

Meningopneumonitis.—This virus was originally isolated in ferrets after inoculation with human throat washings[84] and was named for the characteristic pathology produced in inoculated mice and other experimental animals. Although not obviously associated with birds it resembles ornithosis virus very closely and was found in the experiments of Hilleman[73] to be apparently antigenically identical to that virus.

"Human Pneumonitis."—Several strains of virus isolated by Eaton and his colleagues (*e.g.* strain SF) from man can be distinguished from the other members of the group on various bases. They appear to be most closely related to psittacosis strains but a difference in pathogenicity for the mouse when intraperitoneal inoculation is used has been described.

Lymphogranuloma Venereum.[85]—Although known during the nineteenth century, this disease, also called *lymphopathia venereum*, *lympho-*

[83] Rivers and Berry: Jour. Exp. Med., 1935, *61:*205.
[84] Francis and Magill: Jour. Exp. Med., 1938, *68:*147.
[85] *Cf.* Nelson: *Lymphogranuloma Inguinale*, pp. 365–381.[1]

*granuloma inguinale** and *climatic bubo*, has come into prominence only during the present century, and there is much evidence that it is increasing in incidence in many parts of the world.[86] At first believed to be confined to the tropics and subtropics, it has since been recognized not only in seaports of the temperate regions but in inland cities as well. It has been found in most regions of the United States but is especially prevalent in the South, where the incidence is high in the Negro race. Its venereal transmission makes it more widespread in the lower classes of society, prostitutes in some localities showing a high morbidity.

The primary lesion, occurring upon the genital organs, is usually small and is often overlooked by the patient and not brought to a physician's attention. Extension into the lymphatics produces the typical manifestation of inguinal bubo in the male, while, in the female, lymph nodes within the pelvis are involved. The chronic inflammation in this locality may result in rectal stricture; extensive ulceration in the perineal region and hypertrophic lesions of the external genitalia may also occur.

The Virus.—Successful transmission to animals was first accomplished by Hellerström and Wassén (1930), who found that monkeys proved susceptible to intracerebral inoculation of pus from inguinal buboes. Since then mice also have been found susceptible, and isolation of the virus is now usually accomplished by intracerebral injection of these animals with human material. Monkeys and mice react to intracranial injections with a meningo-encephalitis, and intradermal or subcutaneous inoculation in several species is reported to result in papular lesions in which the virus may be demonstrated. Propagation on several types of tissue culture has been successful and the virus reaches a high titer when grown in the yolk sac of fertile eggs.

The elementary bodies of this virus were first known as the granulocorpuscles of Miyagawa, but it was not until some time later that all the various morphologic elements associated with this infection were described. The developmental cycle has been observed by several investigators, but is best illustrated in a paper by Rake and Jones[87] who also summarize previous reports. The cycle is strikingly similar to that observed for psittacosis virus and described on page 822. Rake and his colleagues have described a soluble antigen associated with this virus, which can be demonstrated by the complement fixation test, and from the same source has come reports[88] of a toxin in emulsions of this virus as well as the related ones, meningopneumonitis, mouse pneumonitis and feline pneumonitis. The toxins have the characteristics of labile endotoxins and antitoxins prepared against them are highly specific.

Immunity.—Venereal lymphogranuloma is unique among the virus diseases in that it may be diagnosed by a skin test. Frei[89] found that intra-

* Not to be confused with *Granuloma inguinale*, another venereal disease. The etiologic agent appears to be the "Donovan bodies," a microorganism of unknown nature. (*Cf.* Anderson: Science, 1943, *97*:560.)

[86] *Cf.* the review by D'Aunoy and von Haam: Arch. Path., 1939, *27*:1032.

[87] Rake and Jones: Jour. Exp. Med., 1942, *75*:323.

[88] *Ibid.*, 1944, *79*:463.

[89] Frei: Klin. Wchnschr., 1925, *4*:2148.

cutaneous administration of heated pus from an infected bubo results in an inflammatory necrotic area at the site of inoculation. The material also induces a febrile reaction when given intravenously. Brains of infected mice have also been used as a source of Frei antigen but at the present time a commercial preparation of infected yolk sac emulsion, marketed under the name "Lygranum," is commonly used.

Reports on the results of the serum neutralization test are not in agreement, but Rodaniche[90] has described a technique, using mice as the experimental animal, which appears to be satisfactory. She used sera from nine persons who had been Frei-tested and found that all possessed neutralizing ability except one from a person with an inguinal lymphadenopathy of three weeks' duration. The serum of thirty-six Frei-negative persons did not neutralize the virus.

Treatment. This agent is the outstanding one of the few viruses whose action is influenced by the sulfonamide drugs. Numerous clinical reports have appeared of the beneficial effect of sulfanilamide and related drugs in human cases, and laboratory experiments[91] indicate that these drugs can influence the course of the infection in mice.

Mouse Pneumonitis. A virus latent in certain mouse stocks has been encountered by several investigators.[92] Continued intranasal passage of lung tissue in such stocks results in an increase in virulence so that the mice die with extensive consolidation of the lungs. Elementary bodies characteristic of this group of viruses are demonstrable in infected tissue smears and the various morphologic elements described for psittacosis and lymphogranuloma venereum, have been observed with this virus. Like lymphogranuloma venereum, mouse pneumonitis virus is adversely affected by sulfonamide drugs. It belongs in this group on other bases as well but is antigenically different from others of the group. Practically, it is a potential hazard in working with other pneumotropic viruses in mice because of the possibility of contamination of the other strain from carrier mice. When using mice for work with other pneumotropic agents one should exert every effort to provide himself with mice free from this virus.

Feline Pneumonitis. In 1942 Baker[93] described the isolation from sick cats of a virus infective intranasally for mice and other animals and for the yolk sac of fertile eggs. The virus, associated with elementary bodies similar to the others of this group, is present in the respiratory tract as well as the ocular and nasal discharges of the cats. Transmission was obtained by contact.

TRACHOMA

Trachoma is a chronic disease of the eye characterized primarily by papillary or follicular hypertrophy of the conjunctiva, with vascularization, or "pannus" formation, in the cornea and secondarily, by cicatrization of the conjunctiva and destructive changes in the lids and cornea. In some cases it produces partial or total blindness. Often, in the early stages and

[90] Rodaniche: Jour. Inf. Dis., 1940, *66*:144.
[91] MacCallum and Findlay: Lancet, 1938, *ii*:136.
[92] Nigg and Eaton: Jour. Exp. Med., 1944, *79*:497; Karr: Jour. Inf. Dis., 1943, *72*:108
[93] *Cf.* Baker: Jour. Exp. Med., 1944, *79*:159.

at intervals during its course, there is acute inflammation accompanied by secondary bacterial infection which is no doubt partly responsible for the inflammatory condition. Although trachoma can be transmitted by contact, ordinary hygienic precautions are sufficient to prevent infection of those in daily association with patients. Affecting a large proportion of the population in some countries, trachoma in the United States is largely confined to some sections of Kentucky, Tennessee, Illinois and Missouri, and to some of the Indian reservations.[94]

Cell inclusions in the conjunctiva are regularly encountered in the human infection, being present with sufficient frequency to provide a simple means of differentiation of early cases from follicular conjunctivitis and other clinically similar conditions. These inclusion bodies have long been regarded as representing the etiological agent of the disease. Halberstaedter and von Prowazek,[95] who first described the cell inclusion, believed it to be a *Chlamydozoon* and classed the disease with smallpox. Later a number of workers came to the belief that these bodies represent "nests" of growing bacteria or cell-reaction products stimulated by bacterial infection.

Hemoglobinophilic bacilli isolated by Williams[96] from cases of trachoma were apparently associated with complicating bacterial conjunctivitis. A small, feebly motile, hemoglobinophilic bacillus, *Bacterium granulosis*, isolated by Noguchi,[97] was the subject of extensive investigations and for a time was widely accepted as the cause of the disease, although general confirmation of the findings of Noguchi and others was lacking.[98]

Since 1930 the chief emphasis in investigation has shifted again to the inclusion body. Transmission of trachoma to monkeys with infective material free of all bacteria has been accomplished with filtrates[99] and with material from testicular passage[100] in animals. In spite of extensive search the characteristic inclusion body has not been found in the experimental disease. It has been emphasized that the elementary bodies of the cell inclusion in man are closely identified with the virus of trachoma. This relationship appears to be even more clearly established from inclusion conjunctivitis, an eye infection of the newborn clinically similar to trachoma and with inclusions which are morphologically like those of trachoma.[101]

The similarity of these inclusions to those of other viruses, especially psittacosis and lymphogranuloma venereum, has been pointed out.[102] The

[94] For a thorough discussion of trachoma, including its history, epidemiology and etiology, see Julianelle: *The Etiology of Trachoma*. The Commonwealth Fund. New York. 1938.

[95] Halberstaedter and von Prowazek: Deut. med. Wchnschr., 1907, *33*:1285.

[96] Williams *et al.*: Jour. Inf. Dis., 1914, *14*:261.

[97] Noguchi: Bull. N. Y. Acad. Med., 2nd Ser., 1927, *3*:295; Jour. Exp. Med., 1928, *48*: Suppl. 2.

[98] *Cf.* Weiss: Jour. Inf. Dis., 1930, *47*:107.

[99] Thygeson: Amer. Jour. Ophth., 1933, *16*:409; Thygeson and Proctor: Arch. Ophth., 1935, *13*:1018; Julianelle and Harrison: Amer. Jour. Ophth., 1935, *18*:133.

[100] Julianelle and Harrison: Amer. Jour. Ophth., 1937, *20*:354.

[101] Thygeson: Amer. Jour. Ophth., 1937, *17*:1019; Tilden and Gifford: Arch. Ophth., 1936, *16*:51.

[102] *Cf.* Rake and Jones: Jour. Exp. Med., 1942, *75*:323.

large inclusions of trachoma and inclusion conjunctivitis give a positive test for glycogen while those of the latter agents do not; otherwise they are morphologically quite similar. It has also been reported[103] that sera of patients with these two eye diseases will fix complement with antigen prepared with the virus of lymphogranuloma venereum, indicating relationship with the psittacosis-lymphogranuloma group.

Claims that the virus of trachoma is rickettsial[104] are not surprising when one remembers the morphologic similarity between rickettsiae and the elementary bodies of psittacosis and related viruses, to which group trachoma has a claim. However, the report that rickettsiae appeared in the intestines of lice after inoculation with trachomatous material and that material from the passage lice was infective could not be confirmed.[105]

Favorable results in the treatment of trachoma and inclusion conjunctivitis with sulfonamide drugs have been reported,[106] but there is incomplete agreement as to whether this is due to an action of the drug upon the virus or upon the secondary bacterial invaders. If the former is true it constitutes another relationship between these viruses and those of the psittacosis lymphogranuloma group, two of which are susceptible to the sulfonamides.

LYMPHOCYTIC CHORIOMENINGITIS[107]

Lymphocytic choriomeningitis is the name given to a specific infection which in man has been included under the general clinical terms *acute aseptic meningitis*, *idiopathic meningitis*, etc. Apparently only a portion of these cases, defined by clinical characteristics, are due to the virus of lymphocytic choriomeningitis,[108] and indeed the latter diagnosis is not justified unless the virus is isolated from the patient, or the development of specific antibody is demonstrated in the serum.

Human Infection. The disease in man begins with a mild upper respiratory infection and some cases do not progress beyond this stage, running their course as an influenza-like illness. In typical cases, a few days after the onset of the prodromal signs, symptoms referable to the nervous system make their appearance. These may consist of sudden severe headache with stiffness of the neck, fever, vomiting, drowsiness, or more severe nervous-system signs. The spinal fluid shows an increased number of cells, most of which are mononuclear. Complications are infrequent and complete recovery is the rule. The virus is present in the blood and spinal fluid during the early stages and has also been demonstrated in the nasopharynx. Serum antibodies make their appearance during convalescence. In a survey[109] in which 1248 human sera were tested, 11 per cent showed

[103] Rake, Schaffer and Thygeson: Proc. Soc. Exp. Biol. Med., 1942, *49*:545.

[104] Busacca: Folia clin. biol. Sao Paulo, 1933, *5*:96; Arch. Ophth., Paris, 1935, *52*:567. Cuenod: Arch. Inst. Pasteur, Tunis, 1938, *24*:86. Rev. internat. trachome, 1936, *13*:9. Cuenod and Nataf: Arch. Inst. Pasteur, Tunis, 1936, *26*:295; Rev. internat. trachome, 1937, *14*:104; Brit. Jour. Ophth., 1937, *21*:309.

[105] Weigl: Centralbl. f. Bakt., I Abt., Orig., 1939, *143*:291; Braley: Arch. Ophth., 1939, *22*:262.

[106] Thygeson and Stone: Jour. Amer. Med. Assoc., 1942, *119*:407.

[107] *Cf.* the review by Blackfan: *Lymphocytic Choriomeningitis*, pp. 684–700.[1]

[108] Baird and Rivers: Amer. Jour. Pub. Health, 1938, *28*:47.

[109] Armstrong and Wooley: Jour. Amer. Med. Assn., 1937, *109*:410.

neutralizing ability. Only 32 per cent of sera from cases diagnosed as aseptic meningitis neutralized the virus. Neutralization tests are positive some weeks after recovery, but positive complement-fixation reactions, dependent upon the presence of a soluble antigen associated with the virus, appear earlier and may prove to be of more value in diagnosis of the disease.

Animal Reservoirs.—This virus was encountered by Armstrong and Lillie in 1934 as a contaminant in monkeys inoculated with the virus of St. Louis encephalitis, and it was the pathological picture in monkeys which gave this infection its name. It was also found to be identical with the agent of a spontaneous disease of mice, described by Traub in 1935,[110] which is transmitted from mother to young in the mouse colony and is maintained by healthy carriers. The virus has since been isolated from human cases,[111] and has been encountered in other monkey and mouse stocks. It has also been found in wild mice trapped in households in which human cases occurred, suggesting that the association of human and murine infection is of etiological significance.[112]

The Virus.—The virus is pathogenic for mice, monkeys and guinea pigs, some investigators finding the latter two species more suitable for primary isolation from man. Inasmuch as this virus occurs naturally in several species of laboratory animal, isolation of the virus by animal inoculation means little unless the animals are known to be free of the infection. Mice with an acute infection are passive and ruffled, or when disturbed exhibit tremors and convulsions. After inoculation by any of several routes virus may be found in the brain and blood and often in urine and nasal secretions. Guinea pigs are susceptible to intracerebral or subcutaneous injections and show fever and loss of weight but no definite neurological signs. The characteristic lesion in experimental animals, as the name indicates, is present in the meninges and choroid plexuses in the form of an inflammation. Monkeys may be infected by a variety of routes and at death virus is found widely distributed throughout the body.

Modification of typical strains has been reported in which repeated passage in guinea pigs has reduced the virulence for mice, and vice versa. Immunization of guinea pigs with the mouse-adapted strain, and with formolized virus, has been reported. A soluble substance associated with this virus can serve as antigen in the complement fixation test.[113] Spleen tissue of infected guinea pigs is a good source of antigen.

The presence of virus in the blood of experimental animals has led to experiments on arthropod transmission of the disease. Coggeshall[114] found that *Aedes aegypti* is able to transmit the infection by first feeding on an infected guinea pig and later upon a normal one. Shaughnessy and Milzer[115] demonstrated similar experimental transmission by the wood tick, *Dermacentor andersoni*, and found that the virus was maintained by the tick

[110] *Cf.* Traub: Jour. Exp. Med., 1936, *63*:533.
[111] Scott and Rivers: Jour. Exp. Med., 1936, *63*:397, 415.
[112] Armstrong, Wallace and Ross: Pub. Health Repts., 1940, *55*:1222.
[113] Smadel and Wall: Jour. Bact., 1941, *41*:421.
[114] Coggeshall: Science, 1939, *89*:515.
[115] Shaughnessy and Milzer: Amer. Jour. Pub. Health, 1939, *29*:1103.

through its various dvelopmental stages and from one geneeration to the next. Guinea pigs were also infected by applying crushed ticks or feces to the scarified skin.

Further experiments[116] showed that bedbugs can take up the virus from infected guinea pigs and transmit the infection to normal guinea pigs while biting. Transmission appears to take place by contamination of the skin of the guinea pig by the bedbug feces which has been shown to contain virus as late as eighty-five days after feeding. Transmission by the bites of monkey lice and of mites was not successful but these arthropods were shown to harbor the virus. Whether any of these findings represent a factor of significance in the natural transmission of this virus is unknown, but it seems likely that arthropods will be found to play some rôle in its epidemiology.

YELLOW FEVER

This disease, at present endemic over large regions in West and Central Africa and in South America, in the past has appeared in epidemic form in seaports of the northern temperate zones on both sides of the Atlantic. Its last appearance in the United States was in 1905 at New Orleans. It is not completely understood why this disease has not spread farther in warm climates, for instance into the Orient, where two important factors for its maintenance are already present, the specific insect vector and a susceptible population. In recent years the facilities for rapid transportation by air and motor highway from the tropics has increased the danger of dissemination, and necessitated strict quarantine measures for travelers from the endemic regions.

The disease when occurring in epidemic form is very severe and carries a high case fatality. The essential damage is to the liver, producing a jaundice in the patient. The lesion, a mid-zonal necrosis in the hepatic lobule (Fig. 233), is pathognomonic of yellow fever. Recovery results in a solid immunity, accompanied by antibody in the serum demonstrable by the serum neutralization test. This antibody apparently persists indefinitely in a recovered patient.

Etiology.[117] Nott in 1848 advanced the hypothesis that insects carried the infection from person to person, and Finlay[118] (1881), convinced that *Aedes aegypti* was the species of mosquito responsible, carried out numerous experiments on human volunteers without convincing results. Carter (1900),[119] in epidemiological studies during an outbreak in Mississippi, defined the limits of the extrinsic incubation period, *i.e.*, the period of time necessary before the "surroundings" of a case became infectious. The period was ten to fifteen days and we now know that this represents the incubation period in the mosquito before it becomes infective. The contributions of Finlay and Carter formed a valuable basis for the experiments undertaken by the American Army Commission (Reed, Carroll, Agramonte,

[116] Milzer: Jour. Inf. Dis., 1942, *70*:152.

[117] *Cf.* Sellards: *Etiology of Yellow Fever and Characteristics of the Infectious Agent*, pp. 713–737.[1]

[118] Finlay: Jour. Amer. Med. Assn., 1901, *37*:1387.

[119] Carter: New Orleans Med. and Surg. Jour., 1900, *52*:617.

Lazear)[120] in Havana, Cuba, at the beginning of the present century. With the aid of human volunteers the Commission established the following important points:

1. Bacteria previously suspected were not etiologically related to yellow fever.
2. The living causal agent of this disease was not cultivable nor visible and was filterable through a Berkefeld candle.
3. The malady was transmissible in nature only by the bites of female *Aedes aegypti* mosquitoes, and was not communicated to nonimmunes through intimate exposure to clothing or bedding contaminated with discharges of patients.

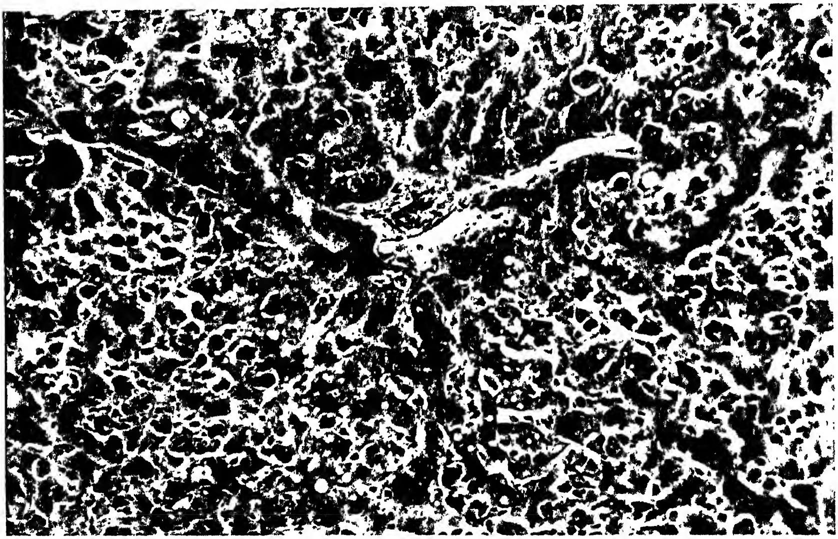

Fig. 233.—Section of liver from a human case of yellow fever. Mid-zonal necrosis is apparent. Hematoxylin and eosin; reduced from × 235. (Obtained through the courtesy of Max Theiler.)

4. A period of about twelve days at summer temperatures was necessary after a mosquito had fed on a patient in the early stage of the disease before it could infect a second individual.
5. The subcutaneous injection of small amounts of blood drawn from a yellow fever patient on the first to third day of the disease was capable of inducing an attack of yellow fever.

The existence of yellow fever in West Africa, where large areas and populations are affected, has long been regarded as a possible menace to the rest of the world. An even wider distribution of this disease in Africa

120. Reed, Carroll, Agramonte and Lazear: Proc. 28th Ann. Meeting, Amer. Pub. Health Assn., Oct. 22, 1900; Reed: Med. Record, 1901, *60:*201; Reed and Carroll: Med. Record, 1901, *60:*641; Amer. Med., 1902, *3:*301; Carroll: Jour. Amer. Med. Assn., 1903, *41:*1341.

is now apparent, and in 1940 an outbreak involving some 15,000 cases occurred in the Nuba Mountains of the Anglo-Egyptian Sudan. Investigators of the International Health Division of the Rockefeller Foundation began a study of the disease in West Africa in 1925. Monkeys of this area were found to be completely resistant to yellow-fever infection, but transmission to Asiatic monkeys (*Macaca mulatta*) was successful, an achievement which made possible an intensive examination into the nature of the disease. Stokes and his colleagues[121] concluded that a filterable virus is the cause of the malady and in general confirmed by means of animal experimentation the work of the commissions of 1900 to 1906. Their experiments were repeated and their results confirmed in West Africa[122] and in South America.[123] Subsequently, yellow fever has been investigated by numerous workers in various countries, and many facts concerning the disease and its agent have been discovered.

The Virus.—The virus of yellow fever, one of the smaller viruses, occurs in the serum of infected monkeys in high concentration and may be preserved by drying blood or bits of tissue from the frozen state.[124] Emulsions in physiological saline deteriorate rapidly but the presence of serum exerts a marked stabilizing effect. The virus has been propagated in different types of tissue culture (p. 794).

Neurotropic Virus.—A notable advance was made by Theiler[125] when he adapted the virus to mouse brain by intracerebral passage, resulting in loss of virulence for monkeys and man when given subcutaneously, but still producing encephalitis in monkeys if given intracerebrally. Such neurotropic strains maintain their immunological identity with natural, or viscerotropic, strains and are suitable for use in serum neutralization tests. Thus, the use of mice allows much more extensive investigation of various kinds than did the larger more expensive monkey.

Mosquito Transmission.[126]—The virus is present in the blood of patients during a few hours preceding the onset and for the first three or four days of illness. A mosquito biting at that time takes up virus with its blood meal. Titrations of the virus content of *Aedes aegypti* have shown that an initial drop occurs followed by an increase in virus over the original amount, indicating multiplication within the mosquito. After about twelve days, depending upon the temperature of the environment, virus reaches the salivary glands. The mosquito is then infective and remains so for the rest of its life. A number of other mosquitoes have been shown by experiment to be potential vectors but *Aedes aegypti* is the one which has been of greatest recognized significance.

Control of Yellow Fever.—The findings of Reed and his colleagues, confirmed by other workers in Cuba and Brazil, suggested measures for combating yellow fever in the countries then concerned. Gorgas in Havana

[121] Stokes, Bauer and Hudson: Amer. Jour. Trop. Med., 1928, *8*:103.

[122] Mathis, Sellards and Laigret: Compt. Rend. Acad. Sci., 1928, *186*:604.

[123] Aragoa: Jour. Amer. Med. Assn., 1928, *92*:550; Davis and Burke: Jour. Exper. Med., 1929, *49*:975.

[124] Bauer and Pickels: Jour. Exp. Med., 1940, *71*:83.

[125] Theiler: Ann. Trop. Med. Parasitol., 1930, *24*:249.

[126] *Cf.* Russell: *Epidemiology of Yellow Fever*, pp. 737–766.[1]

had previously applied extensive general public health measures and succeeded in markedly reducing the general death rate, but had not influenced the incidence of yellow fever. Following the report of the Army Commission he instituted antimosquito measures directed against *Aedes aegypti*. The result was an immediate, sharp decrease in the incidence of the disease. It was found that destruction of *Aedes aegypti* was necessary only in the large urban centers. These appeared to be the endemic foci of the disease and served to supply virus for outbreaks in other cities and localities in communication with them but sometimes at distances of many hundreds of miles. Such measures were taken in the key cities of many localities in Central and South America by representatives of the Rockefeller Foundation in collaboration with local health officers, with the result that yellow fever by 1927 appeared to be all but eradicated from the Western Hemisphere.

Jungle Yellow Fever.—In the years 1928–30, however, it was found that yellow fever was not abiding by the rules laid down from earlier experience. The cleaning up of urban foci no longer sufficed to control the disease. It became necessary in northeast Brazil to institute anti-*aegypti* measures in small towns and even rural districts. These measures proved effective and succeeded in eliminating the disease in that region in 1934. During these years another complexity in other localities was discovered in the form of "jungle yellow fever," a type of the disease found in regions of forests and uncleared land, and, what was more startling, in the complete absence of *Aedes aegypti*. Virus strains from these cases are identical immunologically and otherwise with the classical strains; the difference is epidemiological. Yellow fever in South America is now regarded as primarily a jungle infection with which *Aedes aegypti* is not associated and for which man is not an important source of virus. Soper[127] states that all the cases in South America during 1938 were of the jungle type. It has been postulated that natural reservoirs for the virus are present among the jungle animals, probably monkeys, as epizootics of unknown nature have been noted among monkeys simultaneously with outbreaks of the human disease. Immune sera have been found in monkeys and other jungle animals. Infection of man is regarded as only incidental and not a part of the natural cycle of the infection. Naturally infected jungle mosquitoes (*Aedes leucocelaenus*, *Haemogogus capricorni*, and a *Sabelhine*) have been found[128] but many factors in maintenance and transmission of the jungle infection, including the transmitting vector for man, are not yet known. At the present time cases of jungle yellow fever in man represent potential sources of virus for the start of epidemics in urban areas where *Aedes aegypti* are found.

Field Survey.—Two laboratory procedures have aided greatly in the survey work conducted by the International Health Division of the Rockefeller Foundation in both South America and Africa. One is the serum neutralization test[129] using mouse-adapted virus. This test is specific and

[127] Soper: Trans. Royal Soc. Trop. Med. Hyg., 1938–39, *32*:297.
[128] Shannon, Whitman and Franca: Science, 1938, *88*:110.
[129] Sawyer and Lloyd: Jour. Exper. Med., 1931, *54*:533.

a neutralizing serum indicates previous infection with the virus. Tests by this method have indicated the presence of yellow fever in regions in which it was thought to have disappeared, and by testing sera from persons of various ages in a community it has been possible to estimate at what date yellow fever was last present there.

The other important tool in field survey has been viscerotomy,[130] a procedure by which a portion of liver is taken postmortem from persons dying less than eleven days after the onset of any febrile illness regardless of the clinical diagnosis. Because of the characteristic lesion of yellow fever, histological examination of these specimens enables one to determine with considerable certainty whether the disease was yellow fever. Many thousands of liver specimens have been examined and the presence or absence of yellow fever determined in regions where it would be otherwise impossible.

Active Immunization.[131] Since the epidemiology of jungle yellow fever does not permit its control by the orthodox methods, resort to active immunization of man has been necessary. For this purpose neurotropic strains of virus which have lost their viscerotropism have been invaluable. At first mixtures of this virus and immune serum were given. At the present time a strain of virus, 17D, which has been maintained in tissue culture through many generations, is being used. Repeated transfer in tissue culture has resulted in a strain of virus whose neurotropism and viscerotropism have both been depressed, and it has proved safe and suitable for human use.[132] Subcutaneous inoculation renders monkeys immune to potent strains, and in both monkeys and man neutralizing antibody appears in the serum. Field tests indicate that immunity in man appears not later than a week after inoculation and that vaccinated persons are protected while unvaccinated persons in the same groups may become infected.

The persistence of antibody is greatly influenced by the age of the individual, its disappearance occurring earlier in younger age groups. In an adult population almost all persons show some degree of neutralizing titer after four years and a herd immunity probably lasts much longer.

The vaccine used is a frozen and dried emulsion of infected chick embryo which is rehydrated with a saline solution before use. About 1,900,000 persons in South America had been immunized with this material by the middle of 1940, and it has also been used extensively in Africa. Beside its use in natives of endemic regions, it is given to persons working with yellow fever or going into areas where it exists.

Post-vaccination Hepatitis.[133]—During 1941 and 1942 several million doses of yellow fever vaccines were supplied to the armed forces of the United States. Early in 1942 cases of jaundice began to be observed in increased incidence, especially in the army, following vaccination by a period of weeks or months. The jaundice was due to hepatitis and some

[130] Soper, Ricard and Crawford: Amer. Jour. Hyg., 1934, *19*:549.
[131] *Cf.* Gordon: *Preparation and Use of Yellow Fever Vaccine*, pp. 767–788.[1]
[132] Soper and Smith: Amer. Jour. Trop. Med., 1938, *18*:111.
[133] Sawyer, *et al.*: Am. Jour. Hyg., 1944, *39*:337; *40*:35.

cases were fatal. Such cases had been observed previously in vaccinated individuals, and although the cause of the jaundice was not fully determined, a change in the virus strain used in preparation of the vaccine was apparently sufficient to remove this complication. Up to the end of 1942 there had been recorded 26,771 cases of post-vaccination jaundice in the army, an estimated over-all incidence of 18 per 1000 vaccinated persons. Most of the cases, however, followed the use of only certain lots of vaccine, the incidence of jaundice produced with some lots reaching 50 to 100 per 1000. Investigation suggested that certain lots of human serum used in making the vaccine were the responsible factor, and with the elimination of serum from the vaccine, cases of jaundice promptly stopped appearing. Since this experience many instances of jaundice following the inoculation of human serum for other purposes have come to light.

Although a final decision as to what factor in the vaccine was responsible for these cases cannot be made, the evidence strongly suggests than an icterogenic agent of infectious nature may be present in some human serums and capable of producing hepatitis in inoculated individuals. Although a number of the donors of serum used in the manufacture of the vaccine had histories of "catarrhal jaundice," the presence of healthy carriers of the icterogenic agent in the population without history of jaundice must be postulated to fit the observed facts.

It seems likely that the agent is similar to that of infectious hepatitis, a clinically and pathologically similar disease, to which attention has been called in recent years by outbreaks occurring in troops.[133a]

DENGUE FEVER

Dengue,[134] or "breakbone fever," is an infectious disease found in warm climates. It is characterized by fever, headache, muscular pains and, in a large fraction of cases, a maculopapular rash. About 30,000 cases occurred in Galveston, Texas, during the summer and fall of 1922. An outbreak occurred in Florida in 1934.[135]

The epidemiology of dengue presents a striking resemblance to that of yellow fever. It is transmitted by the yellow-fever mosquito, *Aedes aegypti*, and also by *Aedes albopictus*. Epidemics occur, as with yellow fever, when there are simultaneously present cases of the disease, large numbers of *Aedes aegypti*, and many non-immune individuals. The best method of prevention seems to be that based on mosquito-control measures. Immunization procedures have not been successful to date.

The virus of dengue is present in the patient's blood during the last day of the incubation period and usually for the first three days of illness. Infective blood when inoculated either intravenously or subcutaneously into susceptible persons produces the disease. The cultivation of the virus

[133a] Cameron: Quart. Jour. Med., 1943, *12*:139.

[134] A valuable monograph on dengue, containing the results of original investigation in the Philippine Islands and a full bibliography, has been written by Siler, Hall and Hitchens, Manila, 1926. For a more recent review see Simmons: *Dengue Fever*, pp. 349–364.[1]

[135] Jour. Florida Med. Assn., 1935, *21*:395.

on the chorioallantoic membrane of chick embryos has been reported.[136] Animals generally used for experiment are considered insusceptible but inapparent infections have been produced in monkeys.[137] Although this finding does not supply a laboratory animal for practical use, it is of epidemiological interest. It was found that monkeys in endemic regions, like many persons, were immune, while monkeys brought in screened cages from other regions were susceptible. This may indicate an animal reservoir of the virus.

While one attack of dengue confers an uncertain degree of immunity, persons residing in endemic areas are often found to be immune when inoculated with the virus. However, second, third and even fourth attacks are known to occur. It was shown in the Philippines by experimental inoculation that a second attack might be produced after an interval as short as fifty-three days.

PHLEBOTOMUS FEVER[138]

This disease, also known as *pappataci fever, sandfly fever* and *three-day fever*, occurs during the hot dry season in many regions of the tropics and subtropics. It is a fever of a short duration with clinical signs resembling influenza but without the signs of inflammation of the respiratory tract. One attack confers immunity for at least four months and probably for a year or more. The disease is transmitted, as far as is known, only by sandflies (pappataci flies, *Phlebotomus papatasii*) that have fed on the blood of patients, although there is a possibility of a type of congenital transmission in the fly. After feeding, the flies become infective on about the seventh day. Bedbugs and mosquitoes (*Culex pipiens* and *Aedes aegypti*) have been reported unable to transmit the infection.

No unquestionably susceptible experimental animal has been found and experiments have necessarily been done on human volunteers. A claim for cultivation of the virus in fertile eggs has not been confirmed in more recent work.

The virus is present in the blood of man in amounts up to 1000 infectious doses per cubic centimeter one to two days before the onset of fever and at twenty-four hours, but not at forty-eight hours, thereafter. Serum remains infectious when stored in the cold or when frozen and dried. Differential filtration tests indicate that the virus has a probable diameter of 25 to 40 mμ.

Experimental transmission in man is accomplished much more readily by intravenous or intracutaneous inoculation than by subcutaneous or intramuscular. Subjects which have received subinfective doses by one of the latter routes have been shown to be immune to subsequent infection. Prophylaxis by control of the vector is difficult because the flies are able to pass through an ordinary mosquito net.

[136] Shortt, Rao, and Swaminath: Indian Jour. Med. Res., 1936, *23*:865.

[137] Simmons, St. John and Reynolds: Philippine Jour. Sci., 1931, *44*:1.

[138] For a general discussion of this disease and a preliminary report of investigations undertaken by an Army Commission see: Sabin, Philip and Paul: Jour. Amer. Med. Assoc., 1944, *125*:603, 693.

ENCEPHALITIS

Encephalitis, or inflammation of the brain, may be caused by a variety of etiological agents. A number of human diseases of obscure nature are also included under this general term, but due to lack of knowledge concerning them, classification of these entities has not been satisfactory. The following is an adaptation from a classification of the human encephalitides due to viruses, and those suspected of having a virus etiology.[139]

- I. Infectious encephalitis
  - 1. Type A. von Economo's, or lethargic type (chiefly sporadic)
  - 2. Type B. (chiefly epidemic)
    - (*a*). St. Louis encephalitis
    - (*b*). Equine encephalomyelitis (several types)
    - (*c*). Japanese encephalitis
    - (*d*). Russian encephalitis
  - 3. Other types.
- II. Post-infectious, or para-infectious encephalitis.

Lethargic Encephalitis.—von Economo described an epidemic of encephalitis which occurred in Vienna during the winter of 1916–17. This marked the beginning of recognized outbreaks of this disease, termed *lethargic encephalitis*, which appeared in many parts of the world. At present this type of encephalitis[140] is endemic in almost every civilized country, but seldom appears in epidemic proportions.

The early outbreaks seemed to be associated to some extent with epidemics of influenza but the etiology of the disease is not yet established. In the quest for a causative agent interest has centered on various bacteria, chiefly neurotropic strains of *Streptococcus viridans*, and to a greater extent upon viruses. In 1920 Levaditi and Harvier isolated from patients a strain of herpes virus which was capable of producing encephalitis in rabbits, and which they considered to be the cause of the human disease. One of the patients was suffering from herpes, however, and the possibility remains that this was the source of the virus. Other workers have obtained similar results, and herpes virus has been found in brain tissue, nasopharynx, spinal fluid and saliva of cases. The successful isolations are relatively few compared to unsuccessful attempts, and doubt is cast on the significance of this virus by the fact that herpes virus has been isolated also from spinal fluid and saliva of persons without signs of either encephalitis or herpes, Whatever the relation of herpes virus to von Economo encephalitis, it is nevertheless apparent that herpes, or herpes-like viruses, can on occasion produce central nervous system infection in man. The B virus from a case of human myelitis (p. 844) and the demonstration of herpes virus in encephalitis of an infant (p. 807) are illustrations.

St. Louis Encephalitis.—Cases of a disease now known as St. Louis encephalitis first appeared in Paris, Illinois, in the summer of 1932, and were diagnosed as von Economo encephalitis. A larger outbreak occurred in the late summer of the following year in the vicinity of St. Louis, Mis-

[139] Report on the St. Louis Outbreak of Encephalitis, Pub. Health Bull. No. 214, 1935.
[140] *Epidemic Encephalitis*, Third Report of the Matheson Commission. Columbia University Press. New York. 1939.

souri, and these cases were recognized as a new clinical entity. The 1932 cases were shown in retrospect to be this new disease. The disease appeared again in St. Louis in 1937, and smaller outbreaks have been observed in other western states. There is indirect evidence from the serum neutralization test that the infection is present in many parts of the United States. An early survey[141] indicated that 36 per cent of contacts and 9 per cent of non-contacts possess neutralizing serum. In an endemic locality (Yakima Valley, Washington) the incidence of positive sera in persons who have resided there some time is near 80 per cent.[142] Outbreaks of this disease have occurred in the summer but disappear with the advent of autumn. In the 1933 outbreak in St. Louis cases appeared first outside the city, and the morbidity rate for the epidemic was actually higher in the rural districts surrounding the city than it was in the urban area into which the disease later moved. There is no evidence that St. Louis encephalitis is spread by milk, water, or food supply, and attention was early turned to the possibility of insect vectors. Experiments with mosquitoes by the first investigators were negative; since then strong evidence has been presented that mosquitoes are the vector (*vide infra*).

The Virus. Muckenfuss, Armstrong and McCordock[139] in 1933 successfully infected monkeys by intracerebral inoculation of brain tissue of a patient who had succumbed to the disease. Shortly afterward Webster and Fite found that mice are susceptible to intracerebral inoculation, and mice have remained the animals of choice for the investigation of this disease. The infectious agent was shown to be filterable and its relation to the human disease was confirmed by serum neutralization tests with the serum of recovered patients, 95 per cent showing protection. Multiplication of the virus occurs in tissue culture and upon the chorioallantois of fertile eggs.[143]

The virus of St. Louis encephalitis may be stored in buffered glycerin in the refrigerator, or in the frozen state. In an emulsion it is somewhat labile at room temperature but deterioration may be minimized by using normal serum in the diluent. It shows partial immunologic relationship to West Nile virus (p. 844) and Japanese encephalitis virus, especially by the complement fixation test.

Reservoir of Infection.—Beside mice, hamsters are susceptible and a number of other animals and birds have been shown to suffer an inapparent infection after inoculation, detectable by the presence of circulating virus in the blood. Among these is the horse, which fact provides another similarity between this virus and those of equine encephalomyelitis (*vide infra*). These observations, together with the evidence from neutralization tests on serum taken in endemic areas, that many wild and domestic mammals and birds have experienced infection with this virus, indicate that there are perhaps many potential reservoirs of infection. The virus has been isolated from mosquitoes (*Culex tarsalis*) captured in an epidemic region, and nine species of mosquitoes (including *C. tarsalis*) in three genera have

[141] Wooley and Armstrong: Pub. Health Repts., 1935, *49*:1495.
[142] Hammon: Jour. Amer. Med. Assoc., 1943, *121*:560.
[143] Harrison and Moore: Amer. Jour. Path., 1937, *13*:361.

been reported capable of experimental transmission.[144] Several other species have been shown to harbor the virus for some time after feeding upon infected blood. All observed epidemics are said to have occurred in the presence of large numbers of mosquitoes, those in the West being associated particularly with irrigated regions. The tick, *Dermacentor variabilis*, is also capable of transmitting this virus.[145]

In summary, the evidence indicates that this disease has an extrahuman reservoir in birds and mammals from which source man is infected. Transmission is effected by mosquitoes and perhaps other arthropods.

Artificial Immunization.—Mice may be immunized by moderate doses of active virus given intraperitoneally and immune serum has been produced in non-susceptible animals such as the rabbit. A formalin-inactivated vaccine, capable of protecting mice against peripheral inoculation of virus (and against intracerebral inoculation if large and repeated immunizing doses are given) has been described.[146] Although the vaccine was shown practicable for use in man, and capable of inducing neutralizing antibody in about one half of the inoculated subjects, no field trials have been reported. A vaccine rendered non-infective by exposure for short intervals to intense ultraviolet radiation has also been reported.[147] In the dosages used it produced a high degree of immunity in mice.

Equine Encephalomyelitis.—During an epizootic of this disease in California in 1930 a filterable virus was recovered[148] from the brains of affected horses, which proved to be quite distinct from other known neurotropic viruses, including the previously described virus of Borna disease, a European encephalomyelitis of horses. Since then equine encephalomyelitis has been described in most regions of the United States and in parts of Canada, in South and Central America, and in Russia and Japan. During 1938, an epizootic year, 184,662 cases were reported in the United States.[149] A severe form of the disease occurs in the eastern part of this country and the "eastern type" virus recovered from these cases is immunologically distinguishable from the originally described less severe "western type." A "Venezuelan type" has also been isolated which is immunologically distinct from the other two.[150] Infection of man has occurred with all three strains.

Animal Susceptibility.—Although these viruses were first observed in horses, thereby supplying the name now universally used, equines are not the only animal host, and probably not the primary one, for these strains. The eastern type has been recovered from the brains of pigeons[151] found suffering from an epizootic at the time equine encephalomyelitis was prevalent in the same region, and several reports[152] have appeared of

[144] Hammon and Reeves: Jour. Exp. Med., 1943, *78*:241.
[145] Blattner and Heys: Jour. Exp. Med., 1944, *79*:439.
[146] Sabin, *et al.*: Jour. Amer. Med. Assoc., 1943, *122*:477.
[147] Levinson, *et al.*: Jour. Amer. Med. Assoc., 1944, *125*:531.
[148] Meyer, Haring and Howitt: Science, 1931, *74*:227.
[149] Pub. Health Repts., 1940, *55*:601.
[150] Kubes and Rios: Science, 1939, *90*:20.
[151] Fothergill and Dingle: Science, 1938, *88*:549.
[152] van Roekel and Clarke: Jour. Amer. Vet. Med. Assn., 1939, *94*:466.

the demonstration of the virus in the brains of pheasants. Possibly other natural hosts will be found since a great variety of animals have been infected by artificial inoculation of the virus. In addition to the usual laboratory animals (mice, rats, guinea pigs, rabbits and monkeys) calves, goats, gophers, pigeons, guinea fowl, young puppies, and several species of wild rodents are susceptible to the western strain.[153, 154] Sheep, pigs and cats are apparently resistant to the western but susceptible to the eastern strains. Using the Argentine strain, immunologically identical with western virus, Remlinger and Bailly[155] found a number of birds susceptible, including the mallard duck and other migratory species, a finding of possible significance in the natural distribution of the virus. Quail, sparrows, chicks, hedgehogs and goats can be infected with the eastern strain.[152, 156] Infection of turkeys, chickens and other birds, manifest only by the presence of virus in the blood stream, has been described.[157, 158] In extensive tests on sera of animals and birds taken in endemic areas neutralizing antibody against the western strain was found in many cases. As in the case of St. Louis encephalitis (p. 836) a potential reservoir of infection probably exists in wild and domestic animals and birds.

The Virus. Equine encephalomyelitis virus has been demonstrated in the blood of horses and guinea pigs during the febrile period preceding the onset of nervous system signs, but not after defervesence and the appearance of paralysis, when it is found in nervous tissue. After subcutaneous or intravenous inoculation in guinea pigs it may be demonstrated in the blood stream and appears to invade the brain by migration through vessel walls.[159] Once in the brain it may follow nerve tracts. The pathogenesis in young mice (fifteen days) is similar, but in slightly older mice (twenty-one days) there is evidence that the virus uses peripheral nerves as a path to the central nervous system.

Early estimations of the size of this virus, by differential filtration and centrifugation, indicated it was between 20 and 40 mμ in diameter. More recent study[160] assigns a diameter of approximately 50 mμ to both the eastern and western strains and electron micrography reveals circular images of uniform size.

A liponucleoprotein complex with an estimated molecular weight of 152 million has been isolated from infected tissues and shown to behave as the virus.[161] The infectivity of tissues may be preserved in glycerin or in the frozen state, and blood serum taken from infected guinea pigs retains its virulence for three months when kept in the refrigerator.[162]

[153] Giltner and Shahan: Science, 1933, *78:*63.
[154] Howitt: Abstr. of Communications, 3rd International Congress for Microbiology, 1939, p. 92.
[155] Remlinger and Bailly: Compt. Rend. Soc. Biol., 1936, *121:*146.
[156] Tyzzer, Sellards and Bennett: Science, 1938, *88:*505.
[157] Ten Broeck: Arch. Path., 1938, *25:*759.
[158] Davis: Jour. Bact., 1940, *39:*42.
[159] King: Jour. Exp. Med., 1939, *69:*675.
[160] Sharp, *et al.:* Arch. Pathol., 1943, *36:*167.
[161] Taylor, *et al.:* Jour. Inf. Dis., 1943, *72:*31.
[162] Howitt: Jour. Inf. Dis., 1934, *55:*138.

Infectivity for Man.—In the late summer of 1938 while equine encephalomyelitis was prevalent in Massachusetts and Rhode Island, an epidemic of encephalitis comprising approximately forty cases, mainly in children, appeared in the same region. From the brain tissue of several cases coming to autopsy a filterable virus was recovered[163] by mouse inoculation which, from clinical, histological and immunological evidence in experimental animals, proved to be the eastern type of the horse virus. Human infection with equine encephalomyelitis had, in fact, been suspected as early as 1932 by Meyer, who observed an unusual type of encephalitis in three men who were in close contact with horses infected with the western type of the disease. Human infection with the western type was definitely proved by isolation[164] of the virus from man during an epidemic in California in 1938. Since then epidemics have been recognized in various parts of the West, often in conjunction with cases of St. Louis encephalitis.

Surveys by means of the serum neutralization test in the region of the Massachusetts epidemic[165] and in various localities where the western type has occurred have furnished further information concerning human infection with this virus. The observations to date indicate that the presence of antibody in man usually results from an attack of the disease, although in some regions neutralizing sera have been obtained from persons with no history of nervous system disease. These findings are in contrast to the appreciable percentages of sera found in the general population capable of neutralizing St. Louis and certain other neurotropic viruses. All of 14 sera from patients with encephalitis in Massachusetts (eastern type) showed neutralizing ability, but 114 sera from contacts and other persons were negative. The early appearance of neutralizing antibodies (six to eight days) in human infection with the equine virus increases the potential value of the serum neutralization test as a diagnostic procedure.

Transmission.—The occurrence of outbreaks of this disease in late summer, as well as other epidemiological observations, suggests insect transmission, and the presence of virus in the blood of horses and experimental animals is compatible with this view. Kelser (1933) demonstrated the experimental transmission of the western strain to guinea pigs and to a horse by the bites of *Aedes aegypti* mosquitoes which had been allowed to feed upon infected guinea pigs. This was confirmed by Merrill and Ten Broeck[166] who also found that the virus multiplies within the mosquito. A number of other species of *Aedes*, as well as the wood tick *Dermacentor andersoni*, have been found capable of transmitting the disease. During an epidemic in the Yakima Valley specimens of *Culex tarsalis* were found infected in nature with the western virus, and epidemiological observations[167] strongly incriminate this species as the agent transmitting the infection in that epidemic: *Culex tarsalis* and two other species have transmitted western virus experimentally in the laboratory.[168] Western virus has also

[163] Fothergill, Dingle, Farber and Connerley: New England Jour. Med., 1938, *219*:411.
[164] Howitt: Amer. Jour. Pub. Health, 1939, *29*:1083.
[165] *Cf.* Fothergill: *Equine Encephalomyelitis*, pp. 661–683.[1]
[166] Merrill and Ten Broeck: Jour. Exp. Med., 1935, *62*:687.
[167] Hammon, *et al.:* Jour. Inf. Dis., 1942, *70*:263 *et seq.*
[168] Hammon and Reeves: Jour. Exp. Med., 1943, *78*:425.

been recovered from assassin bugs (*Triatoma sanguisuga*) collected in an epizootic region, and experimental transmission[169] has been accomplished with these insects. This species may be one of the arthropod vectors postulated for transmission within the animal reservoir.

Artificial Immunization.—Early attempts to induce active immunity showed that guinea pigs could be protected to some degree by a variety of preparations, the efficacy of which depended mainly upon the amount of residual active virus present in the preparation. Shahan and Giltner, and Cox and Olitsky[170] reported successful immunization with formolized preparations in which no active virus could be detected by various tests. An advance was made when this method of inactivation was applied[171] to the virus as cultured in chick embryos. It had previously been found that chick embryos are quite susceptible to this virus, the titer of infected embryo tissue being considerably higher than that of horse or guinea-pig brain. It has been estimated[149] that 3,000,000 horses and mules, or about one-fifth of the total number in the United States, received prophylactic inoculations during 1939. Some of these were no doubt inoculated during the incubation period of the disease. Nevertheless the incidence was reported as 1.2 per 1000 in unvaccinated, and 0.37 in vaccinated horses and mules.

This type of vaccine has also been employed in immunizing laboratory workers, since a number of cases of infection and death have occurred in persons working with this virus in laboratories.

Passive immunization has also been demonstrated. If an immune rabbit serum of high titer is used mice and guinea pigs can be protected from death even when the serum is withheld until a considerable interval after the virus is injected.[172]

Japanese Encephalitis.—The Japanese form of type B encephalitis has been recognized as a clinical entity for many years, first appearing in Japan in epidemic form during the summer and fall of 1871. Since that time there have been numerous outbreaks characterized by occurrence in warm weather, a selection of the higher age groups, and a high fatality rate. These epidemiological features are similar to those of the first outbreak of St. Louis type of encephalitis, and differ from those of von Economo type of encephalitis, which occurs chiefly in the winter months and attacks younger age groups. The summer incidence raised the suspicion of insect transmission in this disease also, and four species of culicine mosquitoes have been found[173] that can be infected by allowing them to bite patients. The virus persists in the mosquito for several weeks as evidenced by the inoculation of mice with suspensions of infected mosquitoes or by allowing the mosquitoes to feed upon mice.

From the brain tissue of a fatal case in the 1934–35 epidemic a virus was recovered by Hayashi which would infect monkeys by intracerebral in-

[169] Grundmann, *et al.*: Jour. Inf. Dis., 1943, *72*:163.
[170] Cox and Olitsky: Jour. Exp. Med., 1936, *63*:754.
[171] Beard, *et al.*: Science, 1937, *87*:490.
[172] Zichis and Shaughnessy: Jour. Amer. Med. Assoc., 1940, *115*:1071.
[173] Mitamura, *et al.*: Trans. Soc. Path. Jap., 1937, *27*:573; *ibid.*, 1938, *28*:135.

jection. Later other workers established infection in mice with other strains of the virus.[174] The various strains of Japanese encephalitis have been shown to be identical, but in spite of the epidemiological and clinical similarity between this disease and St. Louis encephalitis, the two viruses differ antigenically.[175] Nevertheless there is some minor antigenic relationship,[176] these two agents together with West Nile virus (p. 844) forming a subgroup of neurotropic viruses. A difference between Japanese and St. Louis virus is seen also in the range of susceptible animals. A severe infection occurs in monkeys with the Japanese type, while the reaction in these animals to St. Louis virus is mild or absent. In sheep the latter virus is innocuous but Japanese virus produces a severe infection.

A formalin-inactivated vaccine which protects mice against the experimental disease has been described in conjunction with a similar preparation against St. Louis encephalitis.[145]

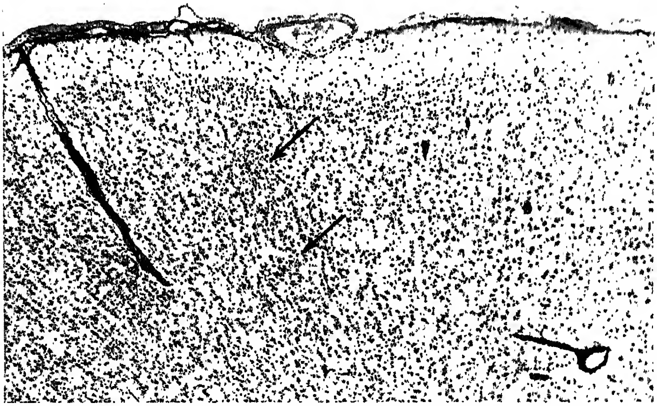

Fig. 234.—Section from the cerebral cortex of a monkey dying with Japanese encephalitis. Cellular infiltration within the meninges and around the blood vessels is apparent. Arrows point to small areas of inflammation within the brain tissue. Hematoxylin and eosin; × 35.

Russian Encephalitis.[177]—(Synonyms: Vernal, Spring-Summer, Forest-Spring, and Tick-Borne encephalitis.) This form of encephalitis was recognized in 1937 among residents of the far eastern provinces of the Soviet Union. It has a seasonal incidence, occurring from May to August, is found in wooded districts and attacks especially persons who do forestry work. It is transmitted by the bite of infected ticks, especially *Ixodes persulcatus*, and congenital transmission of the virus occurs in this tick. On this score as well as by antigenic relationship it resembles louping ill (p. 843), a tick-transmitted virus disease of sheep, but otherwise the epidemiological

[174] Kawamura, *et al.:* Kitasato Arch. Exp. Med., 1936, *13:*281.
[175] Webster: Jour. Exp. Med., 1938, *67:*609.
[176] Casals: Jour. Exper. Med., 1944, *79:*341.
[177] Solowjow: Acta Med. U.S.S.R., 1940, *3:*484; see abstract in Bull. Hyg., 1942, *17:*216.

observations are very similar to those of equine encephalomyelitis (p. 838) and St. Louis encephalitis (p. 836). Numerous forest rodents and domestic animals in endemic regions have yielded neutralizing sera, some have been found naturally infected and others have been proved susceptible by inoculation. There thus appears to be a widespread animal reservoir in addition to the tick. Many persons in endemic regions have antibody in their serum without a history of the disease, indicating that unrecognized infections occur in man.

Successful protection of man in an endemic area has been reported[178] by the use of a formalin-inactivated vaccine.

Other Types of Infectious Encephalitis.—*Australian X Disease.*—In 1917 and 1918 a disease resembling Japanese encephalitis appeared in epidemic form in Australia. An agent which appeared to be a filterable virus was isolated and found to be infectious for monkeys, sheep, cattle and horses. Unfortunately it was not possible to maintain the virus indefinitely, and thorough investigation has not been possible. Similar epidemics occurred in 1922 and 1926 but further strains were not isolated. The agent found in this disease had striking similarities with the virus of louping ill.

Louping Ill.[179]—This is a disease primarily of sheep, occurring in Scotland and the northern counties of England, and characterized by encephalomyelitis. The virus is infectious also for monkeys and mice, resembling in this respect the agent of Japanese encephalitis. Perdrau[180] has compared histological material from louping ill and the Australian disease and found the lesions very similar, the characteristic change in each case being necrosis of the Purkinje cells of the cerebellum. This lesion is apparently responsible for the ataxia seen in infected monkeys and sheep. Another similarity to the Australian virus is seen in the infectivity of louping ill for cattle and horses.

Louping ill is transmitted in nature by a tick, *Ixodes ricinus*, and the virus is present in the blood stream during the febrile stage. Animals are susceptible to intranasal inoculation, and several infections in laboratory workers engaged in study of louping ill have been attributed to this virus.[181] These cases were of varying degrees of severity, and although louping ill virus was not isolated from the cases, the diagnosis was made by means of the neutralization test, using serum taken after recovery.

This virus has a definite antigenic relationship to the virus of Russian encephalitis and it is noteworthy that both are transmitted by ticks.

B Virus.—A filterable agent, now known as B virus, was isolated from the central nervous system of a laboratory worker who died of an ascending myelitis with visceral necrosis, following a bite on the hand by an apparently normal monkey. The virus is infectious for rabbits and monkeys and is capable of producing lesions in skin, viscera, and nervous tissue. In this respect it resembles the virus of pseudorabies of animals, and its

[178] Smorodintseff, *et al.:* Arch. ges. Virusforsch., 1941, 2:1.
[179] *Cf.* Fothergill: *Louping Ill*, pp. 701–712.[1]
[180] Perdrau: Jour. Path. Bact., 1936, *42*:59.
[181] Rivers and Schwentker: Jour. Exp. Med., 1934, *59*:669.

affinity for skin and nervous tissue, as well as its behavior in the rabbit relates it to the virus of herpes. All these three agents produce intranuclear inclusions. Although not antigenically identical with herpes and pseudorabies, B virus has shown partial immunological relationship to both.[182] A number of normal monkey sera have been found capable of neutralizing B virus and it is postulated, therefore, that natural infections occur in monkeys.

West Nile Virus.—In 1940 the isolation of a virus was reported[183] from the blood of a native of Uganda suffering from a febrile illness. During convalescence the patient developed antibody against the isolated agent. Serum tests on natives indicate the presence of this virus in several regions of Africa. The virus is neurotropic, infective for mice and rhesus monkeys and has an estimated particle diameter of 21 to 31 mμ. It is antigenically related to the viruses of St. Louis and Japanese encephalitis.

Relationships of the Viral Encephalitides. Investigation in this group of viruses has been intense in recent years and certain relationships and generalities are becoming apparent. It is noteworthy that a number of these agents are transmitted by arthropods (eastern and western equine encephalomyelitis, St. Louis and Japanese encephalitis by mosquitoes and perhaps other vectors; Russian encephalitis and louping ill by ticks) and that most of these at least are primarily diseases of animals with infection of man being only incidental.

Antigenically the relationships appear to depend upon what test is being used.[184] Thus the St. Louis, Japanese and West Nile viruses are related when tested by the complement-fixation test or by the neutralization technique if serum of sufficiently high titer is used. But when cross-resistance tests were performed on actively immunized animals no relationships were revealed. The equine encephalomyelitis strains stand apart antigenically, as far as is known, as is also the case with the agents of the other neurotropic diseases discussed below, poliomyelitis and rabies.

Laboratory Diagnosis.—This consists either of (1) isolation of the agent in a suitable experimental animal from blood, spinal fluid or nervous tissue, or (2) demonstration of an increase in serum antibody during convalescence. A serum specimen should be taken as early as possible during the illness to compare with one or more specimens taken during convalescence. The neutralization test may be used but a complement fixation technic[185] is also available.

Postinfectious Encephalitis.—This is a central nervous system disturbance, seen only rarely, occurring after various infections such as measles, varicella, influenza and vaccinia. Antirabies prophylaxis is sometimes followed by a similar condition. Although this clinical entity has been called encephalitis, the characteristic lesion is not an inflammation. The predominant pathologic change in the nervous tissue is perivascular

182 Sabin: Brit. Jour. Exp. Path., 1934, *15*:248.
183 Smithburn, *et al.*: Am. Jour. Trop. Med., 1940, *20*:471.
184 Casals: Jour. Exp. Med., 1944, *79*:341.
185 Casals and Palacios: Jour. Exp. Med., 1941, *74*:409.

demyelinization, a lesion quite different from those due to the known neurotropic viruses.

The etiology of these cases is obscure, but since they seem to follow virus infections, it has been suggested that they are due to virus strains which have suddenly gained the ability to invade the central nervous system. Against this theory are the facts that efforts to recover virus from the brain of fatal cases have failed, that the lesions, as mentioned above, do not resemble those due to known viruses, and that similar encephalitides are seen after infections due to agents other than viruses. A second hypothesis is that a latent virus has been stirred to activity by the infection, but this seems similarly untenable. A blood-borne toxin and a hypersensitive state have also been suggested as etiological factors. In this connection, it has been reported that repeated injections of normal rabbit-brain tissue into monkeys induces an encephalomyelitis with lesions similar to those seen in human cases.[186]

POLIOMYELITIS (EPIDEMIC INFANTILE PARALYSIS)

This disease[187] was described by Heine in 1840, and several epidemics in the latter part of the nineteenth century were studied intensively. It has since become prevalent in many parts of the world. Primarily a disease of children, the age incidence in recent years has shown a definite rise. The prodromal stage may take the form of a general illness with headache, fever, gastro-intestinal disturbance or upper respiratory disorder. Meningeal signs appear and other evidence of beginning central nervous system involvement may be present. Many cases progress no farther than this *(non-paralytic poliomyelitis)* or may show transient weakness of a member. The classical case goes on to varying degrees of paralysis from which recovery is slow and sometimes is never complete. The essential lesion in a paralytic case is destruction of the large nerve cells in the anterior horn of the spinal cord (Fig. 237). These cells give rise to the motor fibers of the peripheral nerves and their destruction results in flaccid paralysis. Inflammation in the region attacked is evidenced by congestion, cellular infiltration of the grey matter of the cord, perivascular cellular infiltration (illustrated for another neurotropic virus infection in Fig. 234), and phagocytosis of the necrotic motor cells (neuronophagia).

The Virus.—Landsteiner and Popper were able to transmit the disease to monkeys in 1908 by intraperitoneal inoculation of spinal cord from a fatal case. Rhesus monkeys (*Macaca mulatta*) have been used to the greatest extent and are uniformly susceptible to established strains injected intracerebrally, but infection by other routes is somewhat irregular. Several other species may be infected also, and by other routes; *Macaca cynomolgus* apparently surpasses *M. mulatta* in susceptibility. Marked differences in virulence between strains have been noted, and antigenic differences are also present.[188] It was early shown that the causative agent

[186] Rivers and Schwentker: Jour. Exp. Med., 1935, *61*:689.

[187] For historical information and a complete accumulation of knowledge concerning this disease to 1931, see *Poliomyelitis*. Williams & Wilkins Company. Baltimore. 1932.

[188] Trask, Paul, Beebe and German: Jour. Exp. Med., 1937, *65*:687; Kessel, Stimpert and Fisk: Amer. Jour. Hyg., 1938, *27*:519.

would pass bacterial filters and subsequent observations have indicated that it has the usual properties of a filterable virus.

The virus etiology appears to be well established in spite of occasional reports to the contrary. Streptococci of the viridans type have been cultivated from poliomyelitis cords from time to time but these are generally assumed to be contaminants since the balance of evidence does not support any etiological relation between them and the disease.

Susceptibility of Rodents.—In spite of repeated attempts to infect other laboratory animals, monkeys remained for some time the only susceptible host besides man. In 1939 Armstrong[189] reported that a strain of poliomyelitis virus (Lansing strain) which had been established in monkeys, would produce flaccid paralysis in cotton rats (*Sigmodon hispidus hispidus*) and could be transmitted from rat to rat by inoculation of brain tissue.

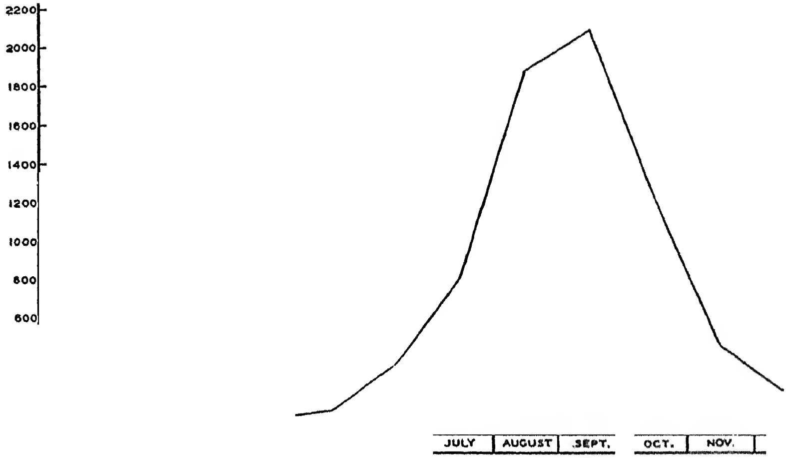

Fig. 235.—The seasonal incidence of poliomyelitis. Averages of reported cases by months for the years 1930 to 1938 inclusive. Data from Supplements to Public Health Reports.

Passage to laboratory mice was then successfully attempted and the mouse-established strain has proved of considerable value in investigations of this virus. Many attempts have been made to establish other monkey strains in rodents but success has been reported in only a few.[190]

Physical Properties.—The virus of poliomyelitis has been described as one of the smallest, its size lying between 8 and 12 mμ when determined by differential filtration. This method assumes, however, that the particle under measurement is essentially spherical in shape. There is a possibility that the virus of poliomyelitis is cylindrical or filamentous in form with the smaller dimension being 12 to 15 mμ and the other much greater.

[189] Armstrong: Pub. Health Repts., 1939, *54*:1719.

[190] Jungeblut and Sanders: Jour. Exp. Med., 1940, *72*:407; Toomey and Takacs: Proc. Soc. Exp. Biol. Med., 1941, *46*:22; Schlesinger, Morgan and Olitsky: Science, 1943, *98*:452.

Gard[191] has described the isolation of such a component from human poliomyelitic stools and from the brains of mice infected with the closely related Theiler's virus (p. 852). Both he and Melnick[192] have observed such filaments in concentrated preparations of virus when viewed with the electron microscope. The fact that a similar component can be isolated from normal stools (although not from normal brain tissue) makes the interpretation of these findings uncertain.

The virus activity of tissue emulsions can be precipitated out with $(NH_4)_2SO_4$ and concentrated by appropriate procedures. The evidence suggests that the virus activity resides in a protein fraction.[193] Compared with certain others, the virus of poliomyelitis is relatively stable. It remains active over a wide range of hydrogen ion concentrations and in the presence of ether. It is resistant to x-rays and sonic vibrations, but relatively susceptible to heat, ultraviolet light and oxidizing agents. It may be stored for long periods of time in 50 per cent glycerol in the refrigerator.

Observations upon infected monkeys indicate that poliomyelitis virus travels by way of nerve tracts. This is true in the case of inoculation of peripheral nerves as well as within the central nervous system in which migration of virus has been traced both by animal test of various tissues and by histologicalo bservations.

Epidemiology.[194,195] Poliomyelitis occurs both sporadically and epidemically and is most common in the late summer and early autumn (Fig. 235). Peaks of high incidence in the U. S. have occurred in 1916, 1931 and in 1943 when 12,429 cases were reported. In California, the state reporting the greatest number of cases in 1943, the case incidence reached 38.8 per 100,000 inhabitants. The reported case fatality, although varying in different outbreaks, has in general declined since epidemics were first observed. This is due at least in part to increased recognition of mild non-paralytic cases which, of course, influences also the figures for morbidity and percentage of paralytic cases. The case-fatality figures for certain epidemic years in Massachusetts[196] are: 1916, 23.5 per cent; 1927, 14.2 per cent; 1935, 4.4 per cent.

Transmission. The mode of transmission of this disease is uncertain. The early epidemiological observations suggested that transmission takes place by contact with cases and carriers, and it was supposed that the upper respiratory tract is the portal of entry and exit of the virus. The finding of virus in nasopharyngeal washings of cases and contacts,[197] as well as various observations on the experimental monkey disease, supports this theory. A number of investigators[198] have found in monkeys that the virus, after being dropped into the nasal cavity, migrates to the

[191] Gard: Acta Med. Scandinavica, 1943, Supp. 143.
[192] Melnick: Jour. Immunol. Virus Res. & Exp. Chemotherap., 1944, *48*:25.
[193] Racker: Science, 1942, *96*:364.
[194] Paul: "*The Epidemiology of Poliomyelitis*," Infantile Paralysis, a Symposium. The National Foundation for Infantile Paralysis. New York City. 1941. pp. 127–157.
[195] *Cf.* Aycock: *Epidemiology of Poliomyelitis*, pp. 555–580.[1]
[196] Legg: New Eng. Jour. Med., 1937, *217*:507.
[197] Kramer, Hoskwith and Grossman: Jour. Exp. Med., 1939, *69*:49.
[198] Bodian and Howe: Proc. Soc. Exp. Biol. Med., 1939, *41*:540.

olfactory bulbs via olfactory nerve, then to the brain stem by way of central olfactory connections, and eventually to the spinal cord. Its journey through the olfactory bulbs is marked by histological changes which serve to indicate its passage. But when the olfactory bulbs of human cases were examined no consistent evidence was found that the virus used this pathway in man. However, this fact does not nullify the possible significance of virus in the throat. A recent study[199] with improved technique reports the isolation of virus in seven attempts out of fourteen from the oropharynx and tonsillar regions of patients. Using the highly susceptible cynomolgus monkey, infection has been produced by non-traumatic application of the virus to the nose and throat by various procedures.[200] Added evidence that virus may be present in the throat is found in the significant correlation between cases of bulbar poliomyelitis and tonsillectomy performed during epidemics. Apparently the virus enters through the wound and

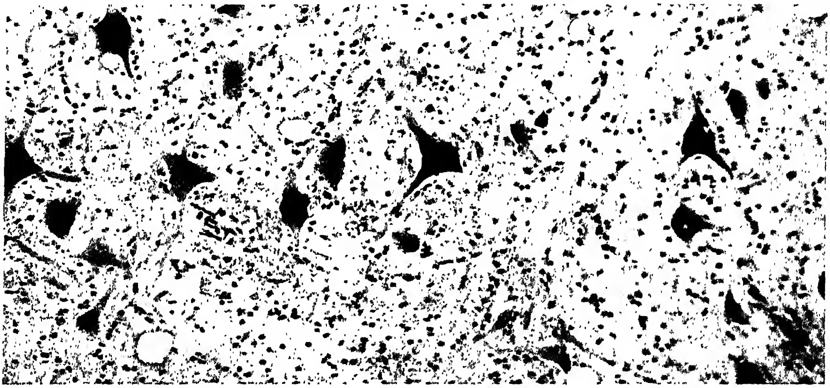

Fig. 236.—Section through the anterior horn of the spinal cord of a normal monkey. This is for comparison with Fig. 237. Hematoxylin and eosin; × 125.

goes directly to the brain stem by way of the nerves supplying the traumatized region.

Epidemiologic studies on small outbreaks in recent years have given evidence that contact with infected cases or carriers was the means of transmission,[201] and virus has been found in the feces of such healthy carriers (*vide infra*).

The seasonal incidence of cases has suggested that insects may play a rôle in transmission. Although the stable fly, *Stomoxys calcitrans*, was at one time incriminated on the basis of successful transmission experiments, repetitions of the experiments produced negative results. Attempts to infect lice, fleas and several species of mosquitoes with laboratory strains have been unsuccessful although the virus was once recovered from bedbugs

[199] Howe, *et al.:* Proc. Soc. Exp. Biol. Med., 1944, *56:*171.
[200] Faber, Silverberg and Dong: Jour. Exp. Med., 1943, *78:*499.
[201] Piszczek, *et al.:* Jour. Amer. Med. Assoc., 1941, *117:*1962; McClure and Langmuir: Amer. Jour. Hyg., 1942, *35:*285.

seven days after feeding. In 1941 several successful attempts to demonstrate virus in flies caught in epidemic areas were reported.[202] These were largely blowflies, houseflies and similar types. Whether flies play a significant rôle in the dissemination and transmission of this virus is not yet apparent but the observed facts are entirely compatible with the theory that the alimentary canal is the portal of entry and exit (*vide infra*).

It has been established in recent years that the virus is present in the stools of patients and healthy contacts, and in sewage from areas where the disease is present.[203] Virus was demonstrated in the tissues of the pharynx and ileum in fatal human cases but rarely from other tissues except those of the nervous system.[204] Experiments with cynomolgus monkeys also give evidence that the virus attacks the pharyngeal and intestinal wall primarily, multiplying there and extending to the central nervous system via the autonomic nerves.[205]

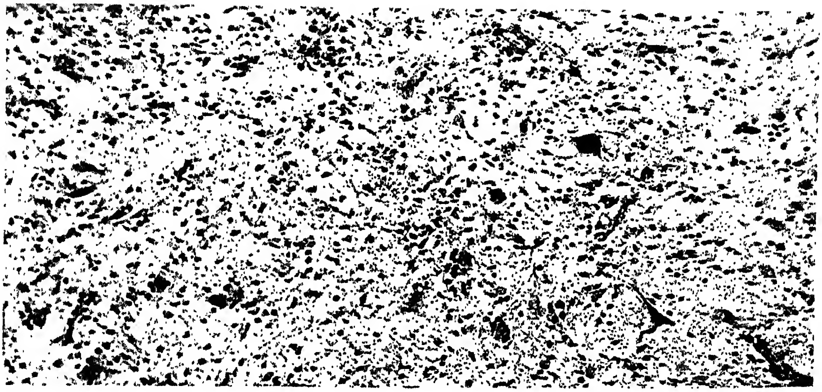

Fig. 237.—Section through the anterior horn of the spinal cord of a monkey paralyzed with poliomyelitis. Destruction of large anterior horn cells has occurred and neuronophagia of the debris is apparent. Compare with the normal spinal cord of Fig. 236. Hematoxylin and eosin; × 125.

The presence of virus in sewage represents a potential source of contamination of water supplies, and the existing reports on the subject suggest that poliomyelitis virus is less readily removed from contaminated waters than are bacterial pathogens. Actually, however, epidemics of poliomyelitis do not have the characteristics of water-borne epidemics and it seems unlikely that contaminated water accounts for very many cases.

Whatever the significance of virus in the stools, in relation to pathogenesis of the disease in man, it is clear that this source of virus must be taken into consideration in any general control measures.

[202] Trask, Paul and Melnick: Jour. Exp. Med., 1943, *77*:531.

[203] *Cf.* Trask, Paul and Vignec: Jour. Exp. Med., 1940, *71*:751; Paul, Trask and Gard: *ibid.*, p. 765; Gard, *ibid.*, p. 779.

[204] Sabin and Ward: Jour. Exp. Med., 1941, *73*:771; *74*:519.

[205] Burnet and Jackson: Australian Jour. Exp. Biol. & Med. Sci., 1940, *18*:361.

Immunity.—Poliomyelitis is an example of a virus disease to which a althy, well-nourished host is more susceptible than is a sickly, mal-ourished one (p. 193). The early clinical observers remarked that strong ell-favored children were usually attacked by this disease. Experiments ith the Lansing mouse-adapted strain and with Theiler's mouse encephalo-yelitis (p. 852) show that well-nourished animals are more susceptible to the virus. The first reports of this work indicated that a deficiency in vitamin B_1 was the factor responsible for resistance but further investigations[206] indicated that an over-all restriction in the diet with adequate B_1 would give similar results.

Active Immunity.—Second attacks occur in man and may be induced experimentally in the monkey. Considering the low incidence in the general population of recognizable poliomyelitis, it becomes difficult to demonstrate statistically that one attack produces any immunity against a second. When a convalescent monkey is reinoculated, extension of the infection in the central nervous system with or without paralysis appears to depend upon how much of the central nervous system was invaded following the first inoculation and whether a different strain of virus was used for the second exposure. It has been demonstrated that different neuron systems are attacked with subsequent injections by different routes.[207] Nevertheless, a severe attack of the disease in a monkey usually renders it resistant to further extension of paralysis following a second injection with homologous virus.

Virus neutralizing substances have been found in the blood of recovered cases (human and monkey) and also in the blood of a surprisingly large proportion of persons with no history of the disease. This last is taken as evidence of widespread subclinical infection. The incidence of neutralizing sera by age groups is compatible with this theory, the percentage of positive neutralizing sera increasing with age in much the same manner as the Schick test in unimmunized populations. Other observations, however, have made the significance of the neutralizing property more doubtful. Some workers have found a considerable proportion of monkey and human convalescent sera devoid of this property and others have found neutralizing serum present at the onset of an attack. It must be remembered in this connection that the neutralization test as performed in monkeys is only very crudely quantitative and because the techniques have varied in different laboratories, optimum conditions for the demonstration of neutralizing property have certainly not always been present. In addition, antigenic differences between strains may account for some of the negative results.[208] The specificity of the neutralizing substance has been doubted by others who declare that the serum acquires virus-inactivating properties as a result of normal physiological maturation. On the other hand, inoculation of monkeys and man subcutaneously with active virus results in an increase in serum titer.

[206] Foster, *et al.:* Jour. Exp. Med., 1944, *79*:221; Rasmussen, *et al.:* Jour. Inf. Dis., 1944, *74*:41.
[207] Howe and Bodian: Jour. Exp. Med., 1941, *74*:145.
[208] Stimpert and Kessel: Amer. Jour. Hyg., 1939, *29*, Sect. B:57.

Active Immunization.—Experiments with monkeys have shown that crude virus or preparations treated in various ways will induce an immunological response when given by intradermal, subcutaneous or intramuscular routes. Only an occasional monkey succumbs to the disease from this procedure, and serum antibody appears in the survivors. There are claims that such injections render the monkeys resistant to inoculation of the virus into the central nervous system. Others have found that such monkeys remained susceptible to intranasal or intracerebral inoculation.[209] The production of neutralizing serum after artificial immunization of monkeys by the procedures that have been tried therefore does not have the same significance as it does in the case of some other infections, *e.g.* equine encephalomyelitis (p. 838). Attempts to immunize children were made with two types of preparation in 1935 and the evidence seems clear that there were twelve cases of poliomyelitis induced in children by the inoculation of vaccine containing active virus.[210] Studies on the serum of inoculated children failed to indicate clearly that inactivated vaccine induced an immunological response, and the use of these preparations was soon discontinued.

Recent reports[211] of experiments using rodent strains of poliomyelitis ndicate that exposure of virus emulsions to ultraviolet light destroys the infectivity but preserves the antigenicity. Most mice were protected against hundreds or thousands of MLD's by previous injections with such vaccines.

Passive Immunization.—Attempts to prevent poliomyelitis in monkeys by prophylactic inoculation of convalescent or immune serum, or to modify the infection by treatment with serum after injection of the virus have been almost entirely unsuccessful. The intracellular habitat of the virus has usually been offered as the reason for the failure of immune serum, present as the result of either active or passive immunization, to arrest the progress of the disease in monkeys. Furthermore, direct contact with central nervous tissue afforded by intracerebral inoculation, and with olfactory nerve endings by intranasal inoculation, allows the virus to reach susceptible cells without being exposed to antibodies present in the serum.

The successful treatment in experimental animals of another neurotropic virus infection (equine encephalomyelitis, p. 838) makes it questionable, however, whether this is the correct explanation. This whole question needs reexamination in the light of our more recent hypotheses regarding the pathogenesis of poliomyelitis. In spite of the negative experiments with monkeys convalescent serum has been used for the treatment of human cases. Various controlled observations fail to show any value in serum treatment.

A non-specific prophylactic measure which appeared hopeful for a time

[209] Kramer, *et al.:* Jour. Immunol., 1937, *31:*167, 183, 191, 199; Olitsky and Cox: Jour. Exp. Med., 1936, *63:*109; Gordon, Hudson and Harrison, 1939, *64:*241.

[210] Leake: Jour. Amer. Med. Assn., 1935, *105:*2152.

[211] McKinstry and Reading: Jour. Franklin Inst., 1944, *237:*71; Milzer, Oppenheimer and Levinson: Jour. Amer. Med. Assoc., 1944, *125:*704.

consisted of the application of zinc sulfate to the olfactory mucosa. This rested upon the theory that virus enters in man via the olfactory nerve endings. Application of zinc sulfate, as well as certain other chemicals, to the olfactory mucosa of monkeys served to protect them temporarily from nasally instilled virus. Field trials[212] in man were disappointing, and it was found that a satisfactory application of the chemical may result in severe damage to the olfactory mucosa.

Encephalomyelitis of Mice.—In 1934 Theiler[213] reported the recovery from the intestines of normal laboratory mice of a neurotropic virus with many characteristics of the strains of poliomyelitis isolated from man. Others have confirmed this discovery and the strain is sometimes called "mouse poliomyelitis," but it should not be confused with the rodent-adapted strains of poliomyelitis which originated in man. It is immunologically different than the Lansing rodent-adapted strain and is not infective for rhesus monkeys.

RABIES (HYDROPHOBIA)

Although the virus of rabies has a broad host range, all mammals and some birds being considered susceptible, rabies is primarily a disease of dogs, cats and wild carnivores. The bites of these animals account for most cases in other mammals and in man,[214] the virus being present in the saliva of rabid animals.

The Disease in Dogs.—Rabies in the dog has a variable incubation period, cases occurring from ten days to as much as a year after exposure. Almost half of the cases, however, have an incubation period of less than thirty days. In the initial stages the dog may show behavior changes and a perverted appetite. A period of increasing excitability may manifest itself first by signs of uneasiness and restlessness, progressing to a stage where the dog runs aimlessly about, snapping at objects in its path or responding with the greatest excitement and fury (furious rabies) to minimal stimuli. Beginning paralysis results in drooling of saliva due to inability to swallow, or the gait may be abnormal. As paralysis increases the dog becomes inactive and stuporous (dumb rabies) and death occurs.

The Virus.—Virus is present in the central nervous system of affected animals, in the salivary glands and saliva, and inconstantly in other glands and in the blood. During the incubation period the dog is not infectious, *i.e.*, virus is not present in the saliva, until a few days (two to eight) before signs of disease appear. Thus a period of observation of ten to fourteen days is sufficient after a dog has bitten to determine whether it was infectious at the time of biting. If signs of rabies do not occur within this period it is safe to conclude that no virus was present in the saliva at the time of biting.

Fixed Virus.—The production of "fixed virus," a term applied by Pasteur to virus that is so exalted in virulence for rabbits by successive intracerebral

[212] Tisdall, *et al.:* Canadian Pub. Health Jour., 1937, *28*:523.
[213] *Cf.* Theiler: Jour. Exp. Med., 1937, *65*:705.
[214] McKendrick: Bulletin of the Health Organization, League of Nations, 1940, *9*:31.

passages that it will produce the death of these animals in six or seven days, is one of the classical examples of variation of a virus (p. 796).

The virus of rabies is one of the larger viruses and is passed through bacteria-retaining filters only with difficulty. It has been propagated in tissue culture[215] and in the chick embryo.[216] It is said to be quickly destroyed when exposed to air and sunlight; drying from the frozen state preserves it for long periods. It is destroyed by many chemical disinfectants but is apparently sufficiently resistant to ether to allow this reagent to be used for the destruction of bacteria in putrefying brain tissue to be tested for the presence of virus.[217]

Transmission to Man.—It is very difficult to estimate the percentage of persons who, not receiving specific prophylaxis, develop rabies after the bite of a rabid animal. The figure is probably in the vicinity of 25 per cent, but the likelihood of rabies developing in a given individual depends upon several factors. A severe wound is more liable to result in infection than a superficial one, but contact of saliva with freshly abraded skin is usually regarded as potentially dangerous. Bites made through heavy clothing are less likely to be followed by rabies than those on bare skin. Virus deposited at the site of a bite reaches the central nervous system by migration along peripheral nerves. This mechanism of pathogenesis doubtless accounts for the relation between site of the bite and length of incubation period, which is longer with bites upon the extremities presumably because the virus has further to travel. The incubation period in man averages six to eight weeks but varies considerably and may be less than a month in the case of bites about the head and neck. Delay in beginning treatment increases the likelihood of a fatal outcome and this is especially true following bites about the head.[214]

Laboratory Diagnosis.—Inflammatory and degenerative changes have been described in the central nervous system of rabid animals, especially in the spinal ganglia, but the most significant effect is the appearance of inclusion bodies named for Negri,[218] who first described them. Although there is no agreement concerning the essential nature of these bodies they have served as a valuable aid in the diagnosis of the disease. They are usually present in the brains of animals dying from infection with "street virus," but are not found in the brains of rabbits infected with "fixed virus." This is ascribed to their failure of development during the shorter incubation period of the latter infection. Smears of brain tissue, or "touch" preparations made by application of a slide to the cut surface of the brain, especially the hippocampus major and the cerebellum, are recommended for the detection of Negri bodies (Fig. 238). These may be stained by any one of several methods; that of Sellers[219] appears to have some advantages over previous methods. Negri bodies are acidophilic masses, usually spherical or oval in shape, lying within the cytoplasm of large ganglion cells (Fig. 221).

[215] Webster and Clow: Jour. Exp. Med., 1937, *66*:131.
[216] Dawson: Science, 1939, *89*:300.
[217] Sulkin and Nagle: Jour. Lab. Clin. Med., 1939, 25:94.
[218] Negri: Ztschr. f. Hyg., 1903, *43*:507.
[219] Sellers: Amer. Jour. Pub. Health, 1927, *17*:1080.

An internal structure can often be seen consisting of basophilic elements within the acidophilic ground substance. Since Negri bodies are not always demonstrable in brains later proved to contain rabies virus (Leach[220] reports that 12 per cent of brains negative for Negri bodies proved to be positive by animal inoculation), the laboratory diagnosis of rabies also involves inoculation of brain tissue into animals. Rabbits or guinea pigs have been used in the past, but there seems to be a distinct advantage in the use of mice, which have been shown to be regularly susceptible to intracerebral inoculation. After inoculation with rabies-positive brain material most mice die within ten to twelve days and Negri bodies can be demonstrated in some cases as early as the fifth or sixth day after inoculation.[219]

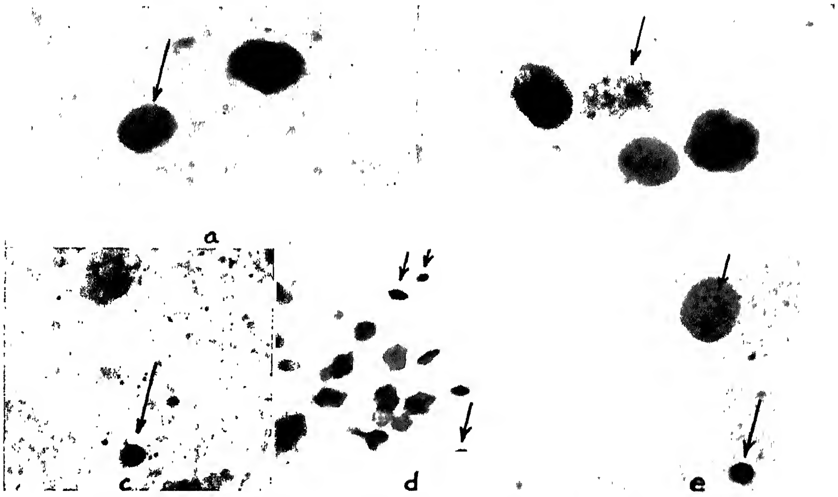

Fig. 238.—Negri bodies in smear from infected dog brains. Sellers stain (p. 853). Photomicrographs *a* and *b* were taken with a filter to bring out the granular nature of the inclusions, and *c*, *d*, and *e* similarly to contrast with the tissue cells. Reduced from the following magnification: *a* and *b*: × 1900, *c*, *d*, *e*: × 800. (From slides supplied through the courtesy of Dr. Joseph Zichis.)

Rabies Prophylaxis.—Pasteur's original method of rabies prophylaxis was to inoculate the exposed person daily with emulsions of rabbit spinal cord, beginning with cord which had been dried for fourteen days and gradually increasing the strength of the dose by using cords which had been dried for shorter and shorter periods. Other methods of using gradually increasing doses of active virus have been introduced as well as the use of virus inactivated by various agents (phenol, chloroform, ether, yatren, dialysis against distilled water, heat, ultraviolet light, antiserum).[221] The commonly used Semple vaccine, inactivated by phenol, is injected daily

[220] Leach: Amer. Jour. Pub. Health, 1938, *28:*162; see also Willett and Sulkin: Jour. Amer. Vet. Med. Assn., 1939, *95:*659.

[221] *Cf.* Kelser: *The Epidemiology and Prophylaxis of Rabies*, pp. 642–660.[1]

for fourteen days. Similar preparations are used in the immunization of dogs.

Webster,[222] in a survey of the field, found that there was little satisfactory experimental evidence regarding the relative efficacy of these various preparations. This is evidently due to the difficulty of obtaining a regular response in animals to a test dose. By means of a mouse test[223] which apparently overcomes this difficulty and runs parallel to satisfactory tests in dogs, Webster has found evidence that chloroform-treated vaccines may be far superior to the phenolized. A mouse test[224] for testing rabies vaccines is now recommended by the National Institute of Health. A vaccine prepared by short exposure of virus emulsion to ultraviolet light of high intensity has given evidence[147] of a much greater immunizing ability than the phenolized vaccines compared with it.

So-called paralytic accidents, of obscure etiology, sometimes follow prophylaxis against rabies. These occurred at the rate of 1 in 7250 treatments at the Pasteur Institutes[214] and there were 7 deaths in 42 cases. This condition, although rare, is a sufficient hazard to avoid giving prophylaxis to persons who have had no real exposure, either to allay their fears or to "play safe."

Since nearly all cases of rabies in man are due to the bite of rabid dogs it is of the utmost importance to check the spread of the disease in these animals and to reduce the incidence of dog bites. The difficulties of this problem and attempts at solution in New York City are discussed by Oleson.[225]

OTHER VIRUS INFECTIONS OF MAN

Molluscum Contagiosum.—This is a contagious disease characterized by superficial benign epithelial tumors of the skin of pearl-like appearance and with a small central depression. The filterability of the active agent appears to be established and histological study[226] indicates that the characteristic inclusion body is composed of elementary bodies in the same manner as those of fowlpox, etc. No susceptible experimental animal has been found.

Warts.[227]—Benign epithelial tumors of several species of animals are known to be filterable. Infectious papillomas of rabbits have been mentioned in connection with other filterable tumors (p. 782). Papillomas of dogs and cattle, and warts (verruca vulgaris) of man are reported to be filterable. Warts of various kinds contain intranuclear inclusion bodies.

Chickenpox (Varicella) and Herpes Zoster.—These are regarded as due to virus agents although the evidence is not complete. Chickenpox is a highly infectious disease of children, usually mild in nature, and characterized by a vesicular eruption. No suitable experimental animal has

[222] Webster: Amer. Jour. Hyg., 1939, *30*:113.
[223] Webster: Jour. Exp. Med., 1939, *70*:87; Webster and Casals: *ibid.*, 1940, *71*:719.
[224] Habel: Pub. Health Reports, 1940, *55*:1473.
[225] Oleson: Pub. Health Repts., 1935, *50*:1087.
[226] Goodpasture and King: Amer. Jour. Path., 1927, *3*:385.
[227] Findlay: Chap. 18 (p. 252), *A System of Bacteriology*, Vol. 7. H. M. Stationery Office. London. 1930.

been found, so that investigation has been handicapped. Elementary bodies, agglutinable in convalescent's serum, are present in vesicle fluid and are regarded as the etiological agent.

Herpes zoster manifests itself by the appearance of dermal vesicles which follow the distribution of one or more sensory, cutaneous nerves, usually of the torso. Animal experiments with this infection have likewise been negative, although human transmission has been accomplished. Complement fixation with convalescent serum has been reported, using crusts of the dried lesions or vesicle fluid as antigen. Paschen in 1933 described the presence of elementary bodies in the vesicles.

Although these two maladies have little in common clinically, an epidemiological association between the two has been noted for many years. Herpes zoster may appear in a person following contact with a case of chickenpox, but more often cases of the latter have been seen to follow contact with herpes zoster. Evidence to explain this association has recently been found in the laboratory. It is claimed that antigens made from the lesions of either disease will fix complement in the presence of convalescent's serum from either herpes zoster or varicella. Amies[228] employed an agglutination technique with pure suspensions of the elementary bodies of both diseases against convalescent's sera from both sources. Some cross-agglutination was obtained but the reactions were not so good as with homologous serum and elementary bodies. The results were interpreted as showing a degree of antigenic similarity between the two agents.

Epidemic Keratoconjunctivitis.—During 1941 and 1942 outbreaks of this eye infection made their appearance in the United States following probable importation of the disease from Hawaii. Although occurring in large numbers in shipyard workers on the West Coast,[229] this feature apparently has no relation to etiology. The disease is characterized by a high degree of infectiousness, relatively little ocular exudate, frequent swelling of the regional lymph nodes, and systemic symptoms, especially headache. The incidence of complications producing impairment of vision has been variable. Sanders[230] successfully isolated a filterable agent in tissue culture in 1942 and was later able to infect mice. The demonstration of neutralizing antibody for this agent in convalescent human sera and the production of a mild case in a human volunteer with cultured virus, fulfilling Koch's third postulate, provide good evidence that the agent isolated is the cause of the disease.

Animal Viruses Transmissible to Man.—In the foregoing sections several virus diseases primarily of animals but also affecting man, have been discussed (equine encephalomyelitis, rabies, louping ill, B virus, psittacosis and others). Investigation of other viruses indicates that animal reservoirs are probably significant (yellow fever, lymphocytic choriomeningitis, St. Louis encephalitis).

Rift Valley fever is a disease of sheep and cattle present in East Africa, probably mosquito-borne. Cases have been noted in sheepherders during

[228] Amies: Brit. Jour. Exp. Path., 1934, *15*:314.
[229] Rieke: Jour. Amer. Med. Assn., 1942, *119*:942.
[230] Sanders: Jour. Exp. Med., 1943, *77*:71.

epizootics and several laboratory infections have occurred.[231] It is described as an influenza-like or dengue-like disease and the virus is present in the blood. Neutralization tests with the sera of natives and Europeans in Africa indicate that the infection is not confined to Kenya, where it was first described, but is present also in other parts of East Africa.

The virus of *foot-and-mouth disease* of cattle is apparently able to attack man. Although many cases have been suspected, only a few have been definitely proved by animal inoculation and examination of serum after recovery. The characteristic lesion in animals is a vesicle upon the skin or mucous membranes, and in the proved cases in man transient vesicles were present upon the hands and feet.

[231] Francis and Magill: Jour. Exp. Med., 1935, *62*:433.

CHAPTER 38

BACTERIOPHAGE

Transmissible lysis of bacteria was discovered by Twort[1] in 1915 and rediscovered by d'Herelle[2] in 1917, and is generally known as the *Twort-d'Herelle phenomenon.* The lytic agent which brings about the dissolution of the bacterial cells is known as *bacteriophage* or, more briefly, as *phage.* The lysis is transmissible indefinitely in series, and the lytic agent is filterable through the usual bacteria-proof diatomaceous earth filters. The resemblance of this transmissible lysis of bacteria to the virus diseases is striking, and the Twort-d'Herelle phenomenon may not unreasonably be regarded as a filterable virus disease of bacteria and the phage as the virus.

Since its discovery this phenomenon has been intensively investigated by a great many workers whose findings and interpretations have been summarized in a series of comprehensive reviews by d'Herelle,[3,4,5] Bordet,[6] Bronfenbrenner,[7,8] Hadley,[9] Burnet[10,11] and Delbrück.[12] Present knowledge of bacteriophage may be considered only briefly here.

Demonstration of Bacteriophagy.—Bacteriophage may be separated from contaminating microorganisms by filtration through Berkefeld N or similar filters. When a drop of the phage-containing filtrate is added to a young broth culture of susceptible bacteria, within an hour or two the culture clears, few if any bacteria can be found on microscopic examination, and those which are present may be the poorly staining "ghost cells." The lysed culture may be filtered and, with the addition of the filtrate to a fresh young broth culture, the phenomenon is repeated and may be continued indefinitely.

Bacteriophage may be found in a variety of places in nature, notably feces and sewage. Filtrates from fecal suspensions or sewage do not always show pronounced lytic activity *per se,* but if the bacterial culture to which

[1] Twort: Lancet, 1915, *ii:*1241.

[2] d'Herelle: Compt. Rend. Acad. Sci., 1917, *165:*373.

[3] d'Herelle: *Le Bacteriophage, son rôle dans l'immunité.* Masson et Cie. Paris. 1921. English trans., Williams & Wilkins Company. Baltimore. 1922.

[4] d'Herelle: *Le Bacteriophage et son Comportement.* Masson et Cie. Paris. 1926. English trans., Williams & Wilkins Company. Baltimore. 1926.

[5] d'Herelle: *Bacteriophage and its Clinical Applications.* Charles C Thomas. Springfield. 1930.

[6] Bordet: Ann. Inst. Pasteur, 1925, *39:*717.

[7] Bronfenbrenner: *Virus Diseases of Bacteria—Bacteriophagy:* Chapter X in Rivers: *Filterable Viruses.* Williams & Wilkins Company. Baltimore. 1928.

[8] Bronfenbrenner: *The Bacteriophage:* Chapter XL in Jordan and Falk: *Newer Knowledge of Bacteriology and Immunology.* University of Chicago Press. Chicago. 1928.

[9] Hadley: Jour. Inf. Dis., 1928, *42:*263.

[10] Burnet: Jour. Path. Bact., 1930, *23:*647.

[11] Burnet: Biol. Rev., 1934, *9:*332.

[12] Delbrück: Advances in Enzymology, 1942, *2:*1.

the filtrate has been added is filtered in turn, and a drop of that filtrate added to a fresh broth culture, etc., complete lysis will appear after a few such "transfers." Filtrates from lysed cultures are generally of high potency and may be diluted to $1:10^9$ or $1:10^{10}$ and still bring about lysis in 1 cc. quantities.

The lytic activity of bacteriophage may also be shown on semisolid media. If the surface of nutrient agar upon which there is a uniform growth of young bacterial culture is streaked with an inoculating needle which has been dipped in phage, clearing of the culture along the needle tract becomes apparent within a few hours. If similar bacterial cultures in Petri dishes are covered with successive dilutions of phage, there will be clearing over the entire surface in the lower dilutions, more dilute phage produces a moth-eaten appearance, and highly diluted phage produces discrete cleared areas in the bacterial growth. These cleared areas, or *plaques*, are analogous to bacterial colonies and the phage may be picked from such "colonies" by

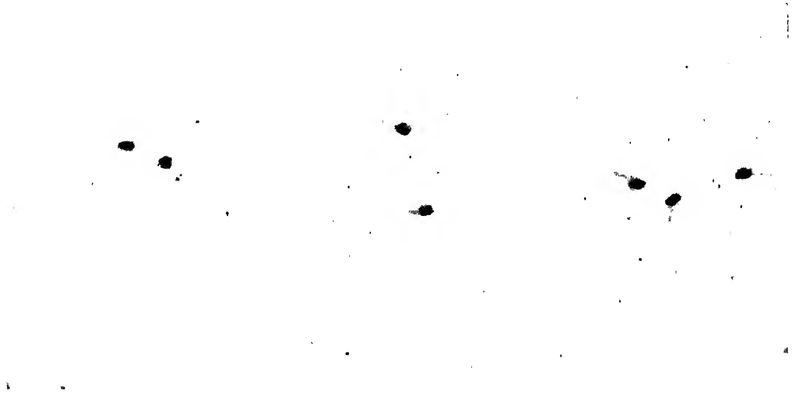

Fig. 239.—Bacteriophage particles of a coliphage. Note the "tails." Electron micrograph; × 24000 (Luria and Anderson).

touching with an inoculating needle and transferring to a fresh young culture of susceptible bacteria. The plaques are variable in size but constant for a given strain of phage; some phages are "large plaque phages" and others are "small plaque phages."

Characteristics of Bacteriophage.—That bacteriophage is particulate is indicated by the formation of plaques, and quantitative studies have shown that the number of plaques decreases in linear relation to increasingly higher dilutions of phage-containing filtrates. Similarly, if equal amounts of phage diluted to almost the limit of activity are distributed in a series of bacterial cultures, the proportion of tubes showing lysis is what would be expected if the lytic agent were particulate. Phage, then, does not exist in true solution. The size of the particles has been measured by filtration through gradocol membranes and by high-speed centrifugation. Pure phages are found to be highly uniform in size; different

phages range in continuous series from 8 to 12 mμ to 50 to 75 mμ in diameter. Examination of electron micrographs of phage and phage lysis has shown that some phage particles tend to be cubical in shape, while others are round and in some cases are characterized by the presence of a "tail," giving an appearance somewhat similar to spermatozoa.[13] (Fig. 239.)

Multiplication.—Because the activity is indefinitely transmissible in series, it will be clear that the phage particles increase in number, and it may be observed that this increase is coincidental with the actual process of lysis. Such multiplication occurs, however, only at the expense of living bacterial cells; phage will not increase in absence of susceptible bacteria nor in the presence of dead bacteria, though it may be shown that phage is absorbed by the dead cells of the susceptible bacterial species. It is generally agreed that multiplication of phage requires the presence of young, actively growing and metabolizing bacteria; it does not occur to any great extent in old cultures or in washed suspensions of "resting bacteria" in non-nutritive buffer solutions.

It was early suggested by d'Herelle that a phage particle enters a susceptible bacterial cell, multiplies and, with the dissolution of the cell, a considerable number of particles are liberated. Such a process has, in fact, been observed directly (see Fig. 241). In keeping with this mode of multiplication, it is found that the phage titer in a lysing culture increases in steps, jumping from none or 1 or 2 to 40 to 60 phage particles in the early stages of lysis. As lysis proceeds, however, the rate of increase is observed to become logarithmic, as might be expected when the individual cellular dissolutions get out of step with one another and thus cancel out the step-like character of the increase.

Specificity.—The bacteriophages are specific in their action, *i.e.*, with respect to the kind of bacteria that are susceptible to lysis. In some instances a relatively broad range of activity may be observed and more than one species of bacteria may be lysed by a single phage; in other cases the activity is highly specific and only a single strain may be lysed. It has been shown, largely through the work of Burnet, that phage specificity is associated with the antigenic structure of the bacteria. This is indicated directly in the case of certain *Salmonella* species; those with common somatic antigens are lysed by a single phage. It is also shown indirectly in the specific inhibition of phage lysis. It has been found that extracts of bacterial cell substance will specifically inhibit lysis; these extracts are inactivated by the homologous antibacterial serum and a precipitin reaction occurs between the two. The precipitating antigen is identical with the phage-inhibiting substance, and the specificity of this substance is determined by a polysaccharide haptene. This haptene, it may be noted, is ineffective in purified form. Furthermore, Delbrück and Luria[14] have shown that if two phages specific for the same bacterium are mixed and added to the bacterial culture, an interference, presumably competitive, is set up and the culture is lysed by one or the other of the phages. From such experiments it is

[13] Luria, Delbrück and Anderson: Proc. Nat. Acad. Sci., 1942, *28*:127; Jour. Bact., 1943, *46*:57.

[14] Delbrück and Luria: Arch. Biochem., 1942, *1*:111, 207.

also apparent that different phages have different "affinities" for the susceptible bacterium.

Metabolism.—Since phage multiplies only in the presence of actively metabolizing bacteria, studies on the physiological activity of phage itself are difficult. Attempts have been made, however, to detect evidences of the metabolism of phage in the absence of bacteria. Bronfenbrenner was unable to detect the evolution of carbon dioxide from filtrates containing phage in high concentration in a specially devised microrespirometer over a period of ten days, and concluded that if respiration occurs it must be at least 10,000 times slower than the respiration of bacterial spores. The same worker showed that, when appropriate corrections were made, the oxygen uptake of lysing cultures was not greater than that of control cultures. Other studies on the reducing power of bacterial cultures have indicated that this ability is not affected in such a way as to indicate a physiological

Fig. 240.—*Bacterium coli* and coliphage after ten minutes' incubation. Note the adsorption of the phage bodies on the surface of the bacterial cells. Electron micrograph; × 17000 (Luria and Anderson).

activity on the part of the phage. It appears, therefore, that bacteriophage does not metabolize to a detectable extent in ordinary ways.

Phage is but little more resistant to bactericidal chemicals and physical influences such as irradiation than the vegetative cells of bacteria. Lytic filtrates may be preserved in the refrigerator for a year or more without significant loss of titer, though the activity of such filtrates shows marked deterioration when stored at room temperature over long periods of time. Some phages are unable to develop in the presence of citrate, *i.e.*, in the absence of calcium, but others are not so affected. Some phages are inactivated by urea

Antigenicity.—The phages are antigenic, and the sera of animals immunized in the usual way with filtrates specifically inhibit the lytic activity. There is also evidence that the phage particles are agglutinated by such antisera. The antisera also contain antibodies to the bacterial substance present in filtrates from lysed cultures. The phages are immuno-

logically heterogeneous, but a number of distinct groups are apparent; it is of some interest that members of the same group may be found in localities geographically widely separated.

There appears to be some correlation between immunological type and the ability to develop in the absence of calcium, resistance to urea, resistance to irradiation, etc., in that the phages belonging to one immunological group exhibit much the same response to such agents but may differ widely in this respect from the more or less homogeneous response of a different immunological group. As yet there has been no formal classification of the phages, but if such is to be made, it would appear that immunological grouping would provide a rational basis.

The Nature of Phage.—It has been urged by d'Herelle from the beginning that bacteriophage is a minute living organism and that there is but one species of this microorganism which is to be designated *Protobios bacteriophagus* or *Bacteriophagum intestinale*. Different races or strains of phage may be regarded as having arisen as a consequence of adaptive responses to environmental influences. A diametrically opposed position has been taken by Bordet, Bronfenbrenner and others, who have maintained that the phage is an inanimate ferment-like substance analogous, perhaps, to lysozyme, but autocatalytic. The adsorption of this ferment on other substances accounts for its apparent particulate nature. A spirited controversy between the exponents of these views was carried on for a number of years and then died down, no solution nor compromise having been reached. It may be noted that, according to a third theory advanced by Hadley and others, phage represents a phase in the life history of the susceptible bacterium and is in the nature of a gene-like substance. This third view has been accepted by only a few.

There is, then, no generally accepted theory of the nature of bacteriophage, and some form of one view or the other noted above is generally supported by individual workers. Whether phage is to be allocated to the category of the living or the inanimate is, perhaps, not too important; at least, such division has become unimportant in the case of the filterable viruses just as the once-debated plant or animal nature of the bacteria has become unimportant. It is reasonable to assume that the phages are filterable viruses since the two agents resemble one another so closely. Such an assumption has the advantage of being non-committal, for the nature of viruses is also unknown.

The Mechanism of Lysis.—The sequence of events that follows the introduction of phage into a culture of susceptible bacteria may be observed under the microscope. Following the entrance of a phage particle the bacterial cell swells, some species to huge proportions and others only moderately or perhaps very little. Within a short time, perhaps twenty minutes, the swollen cell bursts explosively and disappears, leaving only débris visible. Large numbers of phage particles may be seen within the swollen cell, and upon its dissolution are liberated into the surrounding medium. This process is illustrated in Fig. 241.

The mechanism of the swelling and lysis is not clear. Bronfenbrenner and his co-workers have found evidences of proteolysis occurring during

lysis in synthetic media, but the swelling and dissolution of the bacterial cells probably cannot be accounted for on the basis of changes in osmotic pressure and imbibition of water alone; possibly degradation of the structure of the cell is involved also. It is during the process of swelling that multiplication of the phage particles takes place. They have not been seen to divide, however, and the way in which the observed numbers arise is

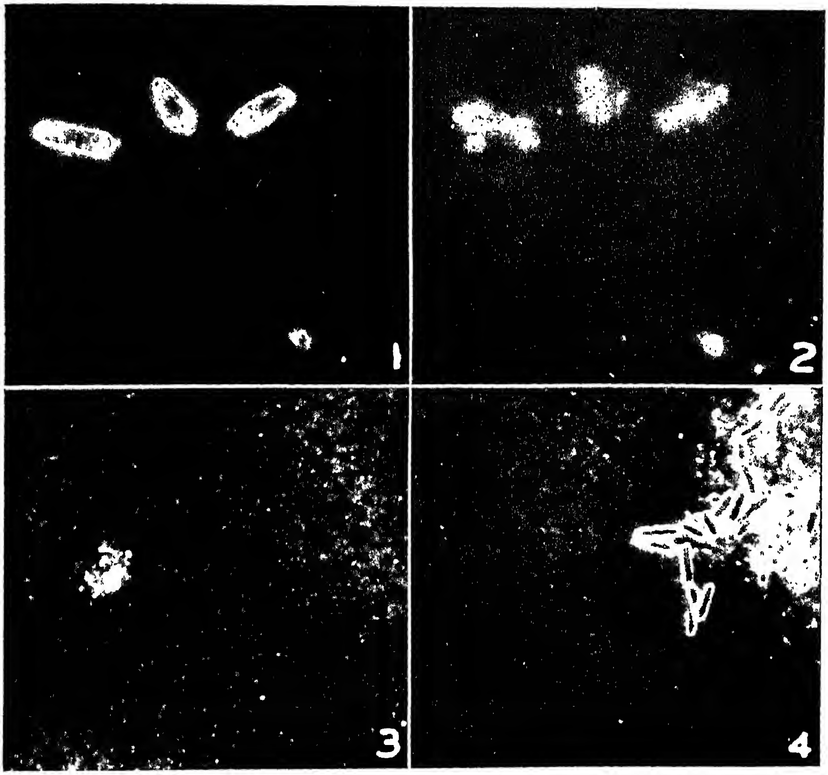

Fig. 241.—The lysis of *Bacterium coli* by phage. *1*, Three bacilli showing phage bodies inside the cells (three hours); × 5000. *2*, The same field five minutes later; the phage particles have been released and the bacilli have collapsed. *3*, Phage particles free in the medium. *4*, Resistant culture growing up in the presence of free phage particles (Merling-Eisenberg).

not known. Krueger and Scribner[15] have found evidence of the existence of a phage precursor in the bacterial cell, and Delbrück and Luria[14] have postulated a "key enzyme" which is associated with phage proliferation.

Effect of Phage on Bacteria.—The most obvious effect of phage on susceptible bacteria is, of course, the lysis of the vast majority of the cells. Preliminary to lysis, however, there is a stimulation of growth of the

[15] Krueger and Scribner: Jour. Gen. Physiol., 1939, 22:699.

bacteria; this effect is transitory and is very soon masked by the developing lytic process. On continued incubation of the lysed culture the few resistant cells remaining multiply with the production of a resistant strain of bacteria. This resistant strain may be unaltered in its immunological character and behavior toward other phages and may differ from the parent strain only in that it is not susceptible to lysis by that particular phage. In many cases, though, changes in other characteristics may accompany the acquisition of resistance; most commonly a certain degree of roughness is apparent in resistant strains derived from smooth parent strains. Phage lysis is a potent method of inducing bacterial variation, but the character, biochemical, serological, etc., of the variants cannot be predicted.

In this connection it is of interest that a symbiotic relation may exist between a phage and a resistant bacterium. Such a relationship is not un-

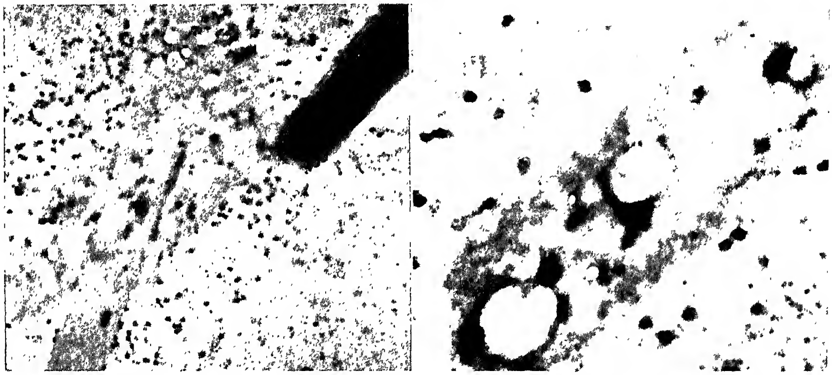

Fig. 242.—Bacteriophage lysis of *Bacterium coli*; electron micrograph. Left, bacterial cell immediately after bursting; note the phage bodies and protoplasmic debris together with the intact cell; × 12500. Right, ghost cell remaining after lysis; note the phage bodies; × 28000 (Luria, Delbrück and Anderson).

common, and phage-carrying strains of bacteria are termed *lysogenic*. The classic example of a lysogenic strain is a strain of *Bacterium coli* isolated by Lisbonne and Carrère[16] and sometimes known as *coli-Lisbonne*. Filtrates from broth cultures of this strain would regularly lyse Shiga dysentery bacilli. The relatively common occurrence of lysogenic strains of bacteria may be of considerable significance to the phenomenon of bacterial variation.

Therapeutic Use of Phage.—d'Herelle has regarded the presence of phage as an important factor in recovery from infectious disease and has urged its therapeutic administration in a variety of infectious processes. The evidence does not, however, support the assertion that phage plays a part in recovery from infectious disease, and it is probable that it is of little or no significance. Phage therapy has been attempted in a variety

[16] Lisbonne and Carrère: Compt. Rend. Soc. Biol., 1922, *86*:569.

of infections, including intravenous injection in septicemia, oral administration in enteric infections such as dysentery, and local application on boils and similar superficial abscesses, and instillation into the urinary bladder in cases of cystitis. The results have been disappointing, however, and it appears that phage therapy is of little value.[17] In some instances in which favorable results have been observed, equally favorable reactions have been secured with sterile broth or phage-free filtrates of bacterial cultures. The beneficial effects of the latter preparations have been ascribed by some to the specific immunizing action of dissolved bacterial cell substance.

[17] *Cf.* Krueger and Scribner: Jour. Amer. Med. Assn., 1941, *116*:2160.

INDEX

Extended discussions are indicated by page numbers in bold-faced type.